United States Environmental Protection Agency | Office of Research and Development Washington, D.C. 20460 | EPA 600-R-06-146 September 2006 www.epa.gov/nhsrc

Technology Evaluation Report

Evaluation of Spray-Applied Sporicidal Decontamination Technologies

EPA 600/R-06/146
September 2006

Technology Evaluation Report

Evaluation of Spray-Applied Sporicidal Decontamination Technologies

By

James V. Rogers, William R. Richter, Young W. Choi,
Emily J. Fleming, Adrienne M. Shesky, Jing Cui,
Michael L. Taylor, Karen B. Riggs, Zachary J. Willenberg, and Harry J. Stone
Battelle
505 King Avenue
Columbus, Ohio 43201

Blanket Purchase Agreement under
General Services Administration Contract No.
GS23F0011L-3

Task Order Project Officer
Joseph P. Wood

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Mail Code E343-06
Research Triangle Park, NC 27711

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development's National Homeland Security Research Center (NHSRC), funded and directed this technology evaluation through a Blanket Purchase Agreement (BPA) under General Services Administration Contract No. GS23F0011L-3 with Battelle. This report has been peer and administratively reviewed and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use of a specific product.

Abstract

The Technology Testing and Evaluation Program (TTEP) is an effort to provide reliable information regarding the performance of commercially available technologies that may have application for homeland security. This effort is an outgrowth of EPA's successful and internationally recognized Environmental Technology Verification (ETV) Program.

As part of EPA's Office of Research and Development, the National Homeland Security Research (NHSRC) rigorously tests technologies against a wide range of performance characteristics, requirements, and specifications. The technology categories of interest include detection, monitoring, treatment, decontamination, and computer modeling. Stakeholder involvement is important to the success of the program. Stakeholders are engaged in identifying and selecting technologies for testing and in developing test plans.

This report presents both quantitative and qualitative results for the spray-applied sporicidal technologies that were evaluated for their effectiveness in decontamination of surfaces. Test coupons that are typical of surfaces found in an office or transportation terminal were selected for the study. The technologies evaluated were:

pH-Amended Bleach (Clorox®)
CASCAD™ Surface Decontamination Foam (Allen-Vanguard)
DeconGreen (Edgewood Chemical Biological Center)
DioxiGuard (Frontier Pharmaceutical)
EasyDecon 200 (Envirofoam Technologies)
Exterm-6 (ClorDiSys Solutions)
HI-Clean 605 (Howard Industries)
HM-4100 (Biosafe)
KlearWater (Disinfection Technology)
Peridox (Clean Earth Technologies)
Selectrocide (BioProcess Associates)

The decontamination efficacy results varied by technology, bacterial spore specie, and coupon material. Following testing, the technology vendors were given the opportunity to review and comment on the draft results.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development (ORD) provides data and scientific support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

In September 2002, EPA announced the formation of the National Homeland Security Research Center (NHSRC). The NHSRC is part of the Office of Research and Development; it manages, coordinates, and supports a variety of research and technical assistance efforts. These efforts are designed to provide appropriate, affordable, effective, and validated technologies and methods for addressing risks posed by chemical, biological, and radiological agents. Research focuses on enhancing our ability to detect, contain, and decontaminate materials in the event of such attacks.

NHSRC's team of scientists and engineers is dedicated to understanding the threat scenarios, communicating the risks, and mitigating the results of attacks. Guided by the roadmap set forth in EPA's Strategic Plan for Homeland Security, NHSRC ensures rapid production and distribution of security related products.

The NHSRC's Technology Testing and Evaluation Program (TTEP) is an effort to provide reliable information regarding the performance of homeland security related technologies. TTEP provides independent, quality assured performance data that is useful to decision makers in purchasing or applying the tested technologies. It provides potential users with unbiased, third-party information that can supplement vendor-provided information and data. Stakeholder involvement ensures that user needs and perspectives are incorporated into the test design so that useful performance information is produced for each of the tested technologies. The technology categories of interest include detection and monitoring, water treatment, air purification, decontamination, and computer modeling tools for use by those responsible for protecting buildings, drinking water supplies and infrastructure, and for decontaminating structures and the outdoor environment.

The evaluation reported herein was conducted by Battelle as part of TTEP. Information on NHSRC and TTEP can be found at http://www.epa.gov/ordnhsrc/index.htm.

Acknowledgments

The authors wish to acknowledge the support of all those who helped plan and conduct the evaluation, analyze the data, and prepare this report. We also would like to thank Dr. Lloyd Larsen, U.S. Army Dugway Proving Ground as well as U.S. Environmental Protection Agency reviewers.

Contents

Tables

Abbreviations/Acronyms

ATCC	American Type Culture Collection
C	Celsius
CFU	colony-forming unit
ClO_2	chlorine dioxide
cm	centimeter
CT	concentration x time
ECBC	Edgewood Chemical Biological Center
EPA	U.S. Environmental Protection Agency
g	gram
hr	hour
L	liter
min	minute
mL	milliliter
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OPP	EPA Office of Pesticide Programs
ORD	EPA Office of Research and Development
PBS	phosphate-buffered saline
ppm	parts per million
psi	pounds per square inch
QA	quality assurance
QC	quality control
QMP	quality management plan
RH	relative humidity
SD	standard deviation
SDF	Surface Decontamination Foam
STS	sodium thiosulfate
TOPO	Task Order Project Officer
TSA	technical systems audit
TTEP	Technology Testing and Evaluation Program

Executive Summary

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) Technology Testing and Evaluation Program (TTEP) helps to protect human health and the environment from adverse impacts of terrorist acts by carrying out performance tests on homeland security technologies. Under TTEP, Battelle evaluated the performance of spray-applied technologies to decontaminate test coupons (1.9 cm by 7.5 cm) prepared from the following materials (typical of surfaces found in an office or transportation terminal):

For testing, coupons were 'contaminated' by spiking with a biological warfare agent - *Bacillus anthracis* Ames – or one of the following, *B. anthracis* Sterne, *B. subtilis* (ATCC 19659), and *Geobacillus stearothermophilus* (ATCC 12980). The spray-applied technologies evaluated and the scope of testing were:

- pH-amended bleach (Clorox® bleach, with water and 5% acetic acid added to obtain pH-amended solution) to inactivate *B. anthracis* Ames, *B. anthracis* Sterne, *B. subtilis*, and *G. stearothermophilus* on test coupons of seven indoor surface materials:
    - Industrial-grade carpet
    - Bare wood (pine lumber)
    - Glass
    - Decorative laminate
    - Galvanized metal ductwork
    - Painted (latex, flat) wallboard paper
    - Painted (latex, semi-gloss) concrete cinder block.
- Ten sporicidal technologies (including four aqueous chlorine dioxide technologies, two hydrogen peroxide technologies, one hydrogen peroxide/peracetic acid technology, two hypochlorous acid technologies and one quaternary ammonium technology) to inactivate *B. anthracis* Ames on glass; the results of the evaluation served as a screening test to down-select four technologies for further evaluation. These four technologies represent four different types of sporicidal chemical formulations that are available
- Four technologies (down-selected from results obtained in the screening tests on glass) to inactivate *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* on test coupons of three indoor surface materials – galvanized metal, carpet, and bare wood.

Testing was performed using a spray application test apparatus developed by Battelle under TTEP. The spray application test apparatus allows for precise control of parameters that could affect the efficacy of spray-applied decontamination technologies, such as mass of spray-applied technology.

The following performance characteristics of sprayed-applied technologies were evaluated:

- Decontamination efficacy
    - —Quantitative assessment of the decontamination efficacy for viable organisms (log reduction)
    - —Qualitative assessment for residual spores on the test coupons
- Qualitative assessment of material surface damage following decontamination.

Results obtained in these tests indicated the sprayed pH-amended bleach inactivated extractable, viable spores from the test coupons. The decontamination efficacy of amended bleach was relatively high (i.e.,

7.2-7.9 log reduction) for hard, nonporous surfaces (glass, decorative laminate, and galvanized metal ductwork) and low (0.28-2.0 log reduction) for the porous surfaces (industrial grade carpet, bare wood, and painted concrete) for *B. anthracis* Ames. For *B. anthracis* Sterne and *B. subtilis*, the results were similar; however, for *G. stearothermophilus*, the log reductions were much lower for hard, nonporous surfaces (0.75-5.90), as well as for porous surfaces (0.02-1.40). Statistically significant differences in the decontamination efficacy were observed when comparing *B. anthracis* to each of the other organisms. *G. stearothermophilus* appeared to be the most resistant to the sprayed, pH-amended bleach.

The results from the qualitative tests using the amended bleach are generally consistent with the results from the quantitative tests. Whereas in the quantitative tests, the amended bleach achieved high decontamination efficiency on hard, non-porous surfaces against *B. anthracis* Ames, *B. anthracis* Sterne, and *B. subtilis*, similar results were seen in the qualitative tests (i.e., few or no positive samples found). Also, whereas the quantitative tests show notably less decontamination efficacy for amended bleach when used against *G. stearothermophilus* on hard surfaces, the qualitative tests showed all positive cultures for this spore type on hard surfaces. No visible damage was observed for any of the test coupons subjected to the sprayed pH-amended bleach.

For the ten technologies, a screening test was used to down-select the four most efficacious technologies that were subjected to more in-depth decontamination efficacy testing. In addition, these four technologies were selected for additional testing because they represent four different types of sporicidal chemical formulations that are available. The screening test evaluated the decontamination efficacy for *B. anthracis* Ames spores on glass coupons. Results obtained in the screening test showed varying decontamination efficacies for the ten technologies ranging from 0.37 to ≥7.8 log reductions. Based on these results, the four down-selected technologies included CASCAD™ Surface Decontamination Foam (SDF), HI-Clean 605, KlearWater, and Peridox. Results for in-depth testing of the down-selected technologies showed that the degree of inactivation varied with respect to the porosity of the test material where greater decontamination efficacy was predominantly observed for hard, non-porous surfaces compared to more porous surfaces. Statistically significant differences in the decontamination efficacy were observed when comparing *B. anthracis* Ames to each of the other organisms. Qualitative assessment of positive liquid cultures resulting from residual viable microorganisms (inoculated spores or endogenous microorganisms) on the test coupons revealed bacterial growth of only the inoculated organism on the streak plates. This reflects improved procedures for sterilizing the coupons (i.e., gamma irradiation) prior to testing that were implemented following the pH-amended bleach decontamination evaluation. There was no physical damage observed for any of the test coupons subjected to the sprayed technologies.

In general, treatment of inoculated coupons with sprayed pH-amended bleach and the four down-selected technologies yielded higher log reductions on non-porous compared to porous materials. However, one notable exception to this is that sprayed Peridox promoted higher log reductions of *G. stearothermophilus* on the porous materials (carpet and wood) compared to the non-porous galvanized metal. The spray-applied CASCAD SDF, HI-Clean 605, KlearWater, and Peridox consistently yielded higher log reductions in *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* spores on industrial carpet coupons compared to pH-amended bleach with the exception of KlearWater for *B. anthracis* Ames. Amended bleach performed the best on galvanized metal, for all spores, with the exception of CASCAD SDF against *G. stearothermophilus*. Moreover, log reductions in *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* spores on bare wood coupons sprayed with Peridox were greater than those sprayed with pH-amended bleach or the other technologies.

Following testing, the technology vendors were given the opportunity to review and comment on the draft results. Three of the four chlorine dioxide based technology vendors expressed concern that the spray system used in testing may not have been operated optimally for their product. An air pressure of 40 psi was used to atomize the liquid, producing a fine mist (10 – 50 micron diameter droplet size). One vendor indicated that this high pressure spray would create relatively small size droplets, leading to increased mass transfer of chlorine dioxide from the liquid to gas phase, thus potentially decreasing the chlorine dioxide concentration in the liquid and rendering it less effective. The other two vendors had made similar comments. Although this phenomenon has not been verified, the reader is thus cautioned about the screening test results reported herein for the aqueous chlorine dioxide based technologies, and that testing of this type of technology at more optimal conditions may be warranted.

1.0 Introduction

NHSRC's TTEP works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, scientists, and permitters; and with participation of individual technology developers in carrying out performance tests on homeland security technologies. In response to the needs of stakeholders, TTEP evaluates the performance of innovative homeland security technologies by developing test plans, conducting evaluations, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure the generation of high quality data and defensible results. TTEP provides unbiased, third-party information supplementary to vendor-provided information that is useful to decision makers in purchasing or applying the evaluated technologies. Stakeholder involvement ensures that user needs and perspectives are incorporated into the evaluation design to produce useful performance information for each evaluated technology.

Under TTEP, Battelle recently evaluated the performance of spray-applied sporicidal decontamination technologies. The primary objective of testing spray-applied sporicidal decontamination technologies was to evaluate their ability to inactivate *Bacillus anthracis* Ames spores and spores of one or more of the following: *Bacillus anthracis* Sterne, *Bacillus subtilis* (ATCC 19659), and *Geobacillus stearothermophilus* (ATCC 12980), on representative indoor surface materials. The spray-applied technologies (note that each technology was applied as liquid droplets, foaming action was not apparent during application) were evaluated as indicated below:

- pH-amended bleach (Clorox® bleach and 5% acetic acid to obtain pH-amended solution) to inactivate *B. anthracis* Ames, *B. anthracis* Sterne, *B. subtilis*, and *G. stearothermophilus* on test coupons of seven indoor surface materials
- Ten sporicidal technologies (including four aqueous chlorine dioxide technologies, two hydrogen peroxide technologies, one hydrogen peroxide/peracetic acid technology, two hypochlorous acid technologies and one quaternary ammonium technology) to inactivate *B. anthracis* Ames on glass; the results of the evaluation served as a screening test to down-select four technologies for further evaluation. The four technologies were selected for additional testing also because they represent four different types of sporicidal chemical formulations that are available
- Four technologies (down-selected from results obtained in the screening tests on glass) to inactivate *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* on test coupons of three indoor surface materials – galvanized metal, carpet, and bare wood.

Testing was performed using a spray system developed by Battelle and specifically used for the present study under TTEP. The spray system allowed for precise-control of parameters that could affect the efficacy of spray-applied decontamination technologies. This spray test system and approach is currently not a standardized method.

These evaluations were conducted according to a peer-reviewed test/QA plan[1] that was developed according to the requirements of the quality management plan (QMP) for the TTEP program.[2] The following performance characteristics of spray-applied technologies were evaluated:

- Decontamination efficacy
  - —Quantitative assessment of the decontamination efficacy for viable organisms
  - —Qualitative assessment for residual spores
- Qualitative assessment of material surface damage following decontamination.

2.0 Technology Description

The table below is a description of the spray-applied sporicidal decontamination technologies evaluated and contact times used based on information provided by the vendor. The information provided in Table 2-1 was not confirmed in this evaluation. Spray-application of the technologies was performed using a spray system developed by Battelle under TTEP and in accordance with the test/QA plan[(1)].

Table 2-1. Technology Information (Vendor Supplied)

Product	Vendor	General Description/ Formula Type	Components	EPA Registration*	Contact Time (min)
Bleach	Clorox®	Sodium hypochlorite	Sodium hypochlorite 5-6% (pH-amended by Battelle by adding acetic acid 5% and water**)	5813-1	10
CASCAD SDF	Allen-Vanguard	Hypochlorite	Sodium myristyl sulfate 10-30%, sodium (C14-16) olefin sulphonate 10-30%; ethanol denatured 3-9%; alcohols (C10-16) 5-10%, sodium sulfate 3-7%; sodium xylene sulphonate 1-5%; proprietary mixture of sodium and ammonia salt along with co-solvent >9%; dichloroisocyanuric acid, sodium salt 48-85%; sodium tetraborate 3-7%; sodium carbonate 10-15%.	None	30
DeconGreen	Edgewood Chemical & Biological Center	Hydrogen peroxide	Potassium molybdate; potassium carbonate; propylene carbonate 25%; H_2O_2 35%, Triton X-100; polyethylene glycol 4-(tert-octyl)phenyl 25%	None	30
DioxiGuard	Frontier Pharmaceutical	Chlorine dioxide	Inerts	None	10
EasyDecon 200	Envirofoam Technologies	Hydrogen peroxide	Hydrogen Peroxide <8%; quaternary ammonium compounds, benzyl-C12-C16 alkyl di-methyl chlorides 5.5-6.5%; diacetin 30-60%	74436-1 and 74436-2	60
Exterm-6	ClorDiSys Solutions	Chlorine dioxide	Inorganic acid 25-35%; sodium chlorite 15-30%; inorganic salt 35-45%; activator 5-10%	70060-19	60
HI-Clean 605	Howard Industries	Hypochlorous acid	Sodium dichlorisocyanurate 11%; trichloro-s-triazinetrione 3%	None	90
HM-4100	Biosafe	Quaternary ammonia	Octadecylaminodimethyltrimethoxysilylpropyl ammonium chloride 84%; chloropropyltrimethoxysilane 15%; dimethyl octadecylamine 1%	None	30
KlearWater	Disinfection Technology	Chlorine dioxide	<0.30% ClO_2 suspended in de-ionized water	None	30
Peridox	Clean Earth Technologies	Hydrogen peroxide	H_2O_2 23-25%; peroxyacetic acid 1-1.4%; acetic acid 1-1.4%; inert ingredients 1-2%	81073-1	10
Selectrocide	BioProcess Associates	Chlorine dioxide	Sodium chlorite 15-40%; activator 55-85%; inert ingredients <2%	74986-4	10

* Registered with the EPA Office of Pesticide Programs (OPP). Registration indicates EPA/OPP has evaluated the pesticide to ensure that it will not have unreasonable adverse effects on humans, the environment and non-target species and has issued a registration or license for use in the United States. Note: No product is registered for use against *B. anthracis*.

** Using procedure recommended by stakeholders, water and 5% acetic acid was added to the household bleach to obtain a pH-amended bleach solution. The solution was prepared using 9.4 parts water, 1 part bleach, and 1 part 5% glacial acetic acid to yield a solution having a mean pH of 6.81 ± 0.15 and a mean total chlorine content of 6,215 ± 212 ppm. This "pH-amended bleach" was evaluated for sporicidal activity.

Below are brief, physical descriptions of the spray-applied technologies (their form, appearance as received from the vendor) and preparation instructions (as supplied by the vendor). The concentrations of active ingredients in the prepared solutions, as reported by the vendor, were not confirmed.

- Bleach – Clorox® bleach purchased in a one gallon container from a local retail store
- CASCAD SDF – The CASCAD™ vehicle/equipment laboratory decontaminant packets are prepared by mixing 9.6 g of GP2100 (decontaminant) and 4.5 mL of GCE2000 (surfactant) in water to yield a final volume of 150 mL
- DeconGreen – Received in a 6.5 gallon pail containing pre-measured component A and two smaller containers, containing pre-measured components B & C. Components B & C are added to component A yielding 5 gallons of activated DeconGreen solution
- DioxiGuard – Two component product that was mixed in equal volumes prior to use. Component A was a chlorine dioxide solution and component B was an inert solution
- EasyDecon 200 – Received in a 6.5 gallon pail kit containing one pre-measured liquid bladder of Penetrator (Part 1), one pre-measured liquid bladder of Fortifier (Part 2), and a pre-measured plastic bottle of Fortifier Booster, (Part 3). The combination of all three pre-measured components yields five gallons of EasyDECON 200 Decontamination Solution finished blend
- Exterm-6 – This chlorine dioxide generating system is a quarter sized tablet that is dissolved into one half gallon of water, yielding a 200 ppm chlorine dioxide solution.
- HI-Clean 605 – Comes as a powder, when mixed with water yields a hypochlorous acid solution. For this testing, a 4% solution was used.
- HM-4100 – Biosafe is an antimicrobial coating (organosilane) that can be applied to metal surfaces, or in the case of plastics and textiles the entire substrate can be treated. The product was used neat.
- KlearWater – Contains 0.15% chlorine dioxide in de-ionized water; the product was used neat.
- Peridox – Comes as a concentrate (24%) which is diluted 1:5 to yield the working solution (4%). The pH of the final solution is 2.7 ± 0.5
- Selectrocide – This product is designed to generate a chlorine dioxide solution in a self contained pouch by adding 2 L of water. Filling the pouch with water initiates the generation of chlorine dioxide from an inner sachet, resulting in a 500 ppm solution (0.05%) of chlorine dioxide dissolved in the water.

3.0 Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the program QMP[2] and the test/QA plan[1] for this evaluation except as noted below. QA/QC procedures are summarized below.

3.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biological safety cabinets) used at the time of evaluation was verified as being certified, calibrated, or validated.

3.2 Audits

3.2.1 Performance Evaluation Audit

No performance evaluation audit was performed for biological agents and organisms because quantitative standards for these biological materials do not exist.

3.2.2 Technical Systems Audit

Battelle QA staff conducted a technical systems audit (TSA) on December 12, 2005 to ensure that the evaluation was being conducted in accordance with the test/QA plan[1] and the QMP.[2] As part of the TSA, test procedures were compared to those specified in the test/QA plan; and data acquisition and handling procedures were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle Task Order Leader for response. In response to the findings of the TSA, a deviation was prepared that accounted for cases where serial dilutions of coupon extracts down to 10^{-5} (not 10^{-7} as stated in the test/QA plan) were performed. The latter approach was used in cases where coupons were treated with a technology that had appreciable (determined during neutralization studies) efficacy and thus dilution plating below the 10^{-5} was not necessary. This approach eliminated unnecessary analyses and conserved resources. TSA records were permanently stored with the TTEP QA Manager.

3.2.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. A Battelle QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

3.3 QA/QC Reporting

Each audit was documented in accordance with the QMP.[2] The results of the TSA were submitted to the EPA (the NHSRC Quality Assurance Manager and the TOPO).

3.4 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in reports. All data were recorded by Battelle staff. The person performing the QC/technical review was involved in the experiments and added his/her initials and the date to a hard copy of the record being reviewed. This hard copy was returned to the Battelle staff member who stored the record.

4.0 Test Results

4.1 pH-Amended Bleach

The decontamination efficacy of pH-amended bleach was evaluated for a biological warfare agent and three additional organisms on seven indoor surfaces. The evaluation followed the EPA-approved *Technology Testing and Evaluation Program Test/QA Plan for Evaluating Liquid and Foam Sporicidal Spray Decontaminants* (Version 1).[(1)] Various structural, decorative, and functional surfaces typically found inside an office building or a mass transit station were used to evaluate the sporicidal decontamination technology. The test surfaces (coupons measuring 1.9 cm x 7.5 cm) are listed below:

- Industrial-grade carpet
- Bare wood (pine lumber)
- Glass
- Decorative laminate (Formica®, white matte finish)
- Galvanized metal ductwork
- Painted (latex, flat) wallboard paper
- Painted (latex, semi-gloss) concrete cinder block.

The decontamination technologies were tested against the biological agent, *B. anthracis* Ames spores. To provide comparative data with the *B. anthracis* Ames, other organisms frequently used in decontamination testing, *B. anthracis* Sterne, *B. subtilis*, and *G. stearothermophilus* spores, were tested in parallel. The following sections summarize the results of these evaluations.

4.1.1 Decontamination Efficacy

4.1.1.1 Quantitative Assessment of the Log Reduction of Viable Organisms

Decontamination efficacy (E) was calculated as the mean log reduction in viable organisms achieved by the decontamination technology. The spraying system treats two coupons at a time; therefore, for the controls and decontaminated samples (for each test material/organism), three separate spray replicates (trials) were employed. This accounted for the six controls and six decontaminated samples (e.g., two coupons x three replicates = six control/decontaminated coupons). The blanks (sprayed with water) were treated separately. The log reduction in viable spores for each individual coupon (E_i) was calculated for each of the six replicates of each material type and biological agent or organism as:

$$E_i = \log_{10} \frac{\overline{N}}{X_i}$$

where $\overline{N}$ was the mean number of viable organisms recovered from the six positive control coupons of a given material type and X_i was the number of viable organisms of a given type recovered from a replicate test coupon (*i*) after decontamination. (Positive controls are spiked with biological agent and run at the same test chamber temperature and RH and analyzed at the same time points as test coupons, but without exposure to the decontaminant technology.) If no viable organisms were recovered from a test coupon after decontamination, the value 1 was substituted for X_i. Since the value 1 is greater than the observed value of zero, the estimate with this substitution becomes a lower bound for the true log reduction. Next, the mean log reduction for a given material type was calculated as decontamination efficacy (*E*) for a given biological agent or organism as:

$$E = \frac{\sum_{i=1}^{n} E_i}{n}$$

where the sum of the log reductions (E_i *values*) was divided by the number of replicates (*n*). The mean log reduction calculated as described above is the decontamination efficacy (*E*).

The decontamination efficacy of amended bleach was high (i.e., 7.2-7.9 log reduction) for hard, nonporous surfaces (glass, decorative laminate, and galvanized metal ductwork) and low (0.28-2.0 log reduction) for the porous surfaces (industrial grade carpet, bare wood, and painted concrete) for *B. anthracis* Ames (Table 4-1). For *B. anthracis* Sterne and *B. subtilis*, the results were similar; however, for *G. stearothermophilus*, the log reductions were much lower for hard, nonporous surfaces (0.75-5.90), as well as for porous surfaces (0.02-1.40). These results are presented in Tables 4-2, 4-3, and 4-4. No viable organisms were detected by the quantitative method in any of the blank samples. The decontamination efficacy results for *B. anthracis* Ames, *B. anthracis* Sterne, *B. subtilis*, and *G. stearothermophilus* spores are summarized in Table 4-5. The decontamination efficacy varied according to the type and porosity of the test material with a greater decontamination efficacy observed for hard, non-porous surfaces compared to more porous surfaces. *G. stearothermophilus* appeared to be the most resistant to the pH-amended bleach.

It should be noted that, in some cases, there were average percent recoveries of <25% of organisms spiked onto control coupons as shown in Table 4-1; however, these values were within the acceptable limits as defined in the test/QA plan. These recoveries of inoculated organisms may be attributed to interactions (adherence or sorption) to the material comprising each test coupon. The recoveries reported herein are similar to the recoveries achieved in previous testing.[3-6] Note also that in the present evaluation as well as previous studies,[3-6] recoveries obtained for *B. anthracis* Ames are generally not the same as the recoveries obtained for the organisms, *B. subtilis* and *G. stearothermophilus.*

The null hypothesis[1] was tested that there were no differences in the efficacy results for *B. anthracis* Ames and the other organisms. Statistically significant differences were observed for the results obtained for five of the seven test materials (Table 4-5). For *B. anthracis* Ames and Sterne as well as *B. subtilis*, no viable spores were detected in extracts from glass and galvanized metal test coupons that were decontaminated with pH-amended bleach. Also, no viable *B. anthracis* Ames and Sterne spores were detected in extracts from decontaminated decorative

laminate coupons following decontamination with pH-amended bleach. For industrial carpet, painted wallboard paper, and painted concrete, the decontamination efficacy for pH-amended bleach inactivation of *B. anthracis* Sterne spores was statistically greater than that of *B. anthracis* Ames. When compared to *B. anthracis* Ames, statistically lower decontamination efficacies were observed in the case of inactivation of *G. stearothermophilus* spores on decorative laminate, galvanized metal, and painted wallboard paper. Statistically lower decontamination efficacy values were obtained for *B. subtilis* spores compared to *B. anthracis* Ames spores on painted wallboard paper. However, statistically greater decontamination efficacy values were obtained for *B. subtilis* spores compared to *B. anthracis* Ames spores on painted concrete. The log reduction values for *G. stearothermophilus* shown in Table 4-5 generally indicate that these spores were more resistant to pH-amended bleach than other spores tested. This trend is especially noticeable upon comparison of the log reduction values obtained for decorative laminate for spores from the four test organisms. The 0.75 log reduction value is not, when one examines the log reduction range (rounded numbers) for the six replicates of this data point (0.84, 0.74, 1.09, 0.50, 0.79, and 0.52) an outlier. *G. stearothermophilus* often, as seen in previous testing (References 3-6), behaves differently.

Table 4-1. Inactivation of *Bacillus anthracis* Ames Spores[a] (pH-Amended Bleach; Ten Minute Contact Time)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Positive Control[b]	8.47×10^7	$4.10 \pm 0.68 \times 10^7$	48.4 ± 8.04	-
Decontaminated[c]	8.47×10^7	$2.14 \pm 0.09 \times 10^7$	25.3 ± 1.02	0.28 ± 0.02
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Positive Control	9.03×10^7	$4.66 \pm 0.80 \times 10^6$	5.17 ± 0.89	-
Decontaminated	9.03×10^7	$1.27 \pm 0.47 \times 10^6$	1.41 ± 0.52	0.59 ± 0.15
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	9.20×10^7	$7.31 \pm 0.93 \times 10^7$	79.5 ± 10.1	-
Decontaminated	9.20×10^7	0	0	≥7.9
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Decorative Laminate				
Positive Control	9.03×10^7	$1.52 \pm 1.22 \times 10^7$	16.9 ± 13.5	-
Decontaminated	9.03×10^7	0	0	≥7.2
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	9.20×10^7	$5.29 \pm 1.18 \times 10^7$	57.5 ± 12.8	-
Decontaminated	9.20×10^7	0	0	≥7.7
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	8.17×10^7	$4.12 \pm 1.10 \times 10^7$	50.4 ± 13.4	-
Decontaminated	8.17×10^7	$4.09 \pm 0.97 \times 10^5$	0.50 ± 0.12	2.0 ± 0.11
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Concrete				
Positive Control	8.47×10^7	$4.42 \pm 0.71 \times 10^7$	52.1 ± 8.38	-
Decontaminated	8.47×10^7	$4.26 \pm 1.36 \times 10^6$	5.03 ± 1.60	1.0 ± 0.12
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated coupon
"-" Not Applicable

Table 4-2. Inactivation of *Bacillus anthracis* Sterne Spores[a] (pH-Amended Bleach)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Positive Control[b]	1.24×10^8	$6.09 \pm 0.56 \times 10^7$	49.2 ± 4.50	-
Decontaminated[c]	1.24×10^8	$9.53 \pm 5.59 \times 10^6$	7.68 ± 4.50	0.88 ± 0.30
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Positive Control	1.24×10^8	$1.46 \pm 0.64 \times 10^7$	11.8 ± 5.16	-
Decontaminated	1.24×10^8	$2.35 \pm 1.46 \times 10^5$	0.19 ± 0.12	1.9 ± 0.31
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	4.73×10^7	$5.96 \pm 3.36 \times 10^6$	12.6 ± 7.11	-
Decontaminated	4.73×10^7	0	0	≥6.8
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Decorative Laminate				
Positive Control	4.73×10^7	$4.03 \pm 2.00 \times 10^6$	8.51 ± 4.23	-
Decontaminated	4.73×10^7	0	0	≥6.6
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	4.73×10^7	$2.27 \pm 1.53 \times 10^7$	47.9 ± 32.4	-
Decontaminated	4.73×10^7	0	0	≥7.4
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	1.16×10^8	$3.11 \pm 0.39 \times 10^7$	26.9 ± 3.37	-
Decontaminated	1.16×10^8	$3.38 \pm 1.00 \times 10^4$	0.03 ± 0.01	3.0 ± 0.12
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Concrete				
Positive Control	1.16×10^8	$7.07 \pm 2.07 \times 10^7$	61.0 ± 17.9	-
Decontaminated	1.16×10^8	$4.23 \pm 2.69 \times 10^5$	0.36 ± 0.23	2.3 ± 0.35
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated coupon
"-" Not Applicable

Table 4-3. Inactivation of *Bacillus subtilis* Spores[a] (pH-Amended Bleach)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Positive Control[b]	1.20×10^8	$2.99 \pm 0.20 \times 10^7$	25.0 ± 1.66	-
Decontaminated[c]	1.20×10^8	$1.98 \pm 0.56 \times 10^7$	16.5 ± 4.63	0.19 ± 0.11
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Positive Control	1.20×10^8	$1.03 \pm 0.19 \times 10^7$	8.57 ± 1.56	-
Decontaminated	1.20×10^8	$3.81 \pm 2.19 \times 10^6$	3.18 ± 1.83	0.49 ± 0.27
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	1.15×10^8	$5.39 \pm 0.49 \times 10^7$	46.9 ± 4.25	-
Decontaminated	1.15×10^8	0	0	≥7.7
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Decorative Laminate				
Positive Control	1.15×10^8	$1.76 \pm 0.19 \times 10^7$	15.3 ± 1.65	-
Decontaminated	1.15×10^8	$3.43 \pm 8.41 \times 10^1$	0	6.90 ± 0.94
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	1.15×10^8	$6.24 \pm 0.85 \times 10^7$	54.2 ± 7.40	-
Decontaminated	1.15×10^8	0	0	≥7.8
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	1.10×10^8	$1.83 \pm 0.84 \times 10^7$	16.6 ± 7.59	-
Decontaminated	1.10×10^8	$9.60 \pm 4.87 \times 10^6$	8.72 ± 4.43	0.33 ± 0.22
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Concrete				
Positive Control	1.10×10^8	$3.61 \pm 1.38 \times 10^7$	32.8 ± 12.5	-
Decontaminated	1.10×10^8	$2.85 \pm 3.75 \times 10^5$	0.26 ± 0.34	2.40 ± 0.50
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated coupon
"-" Not Applicable

Table 4-4. Inactivation of *Geobacillus stearothermophilus* Spores[a] (pH-Amended Bleach)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Positive Control[b]	8.23×10^7	$1.03 \pm 0.20 \times 10^7$	12.5 ± 2.36	-
Decontaminated[c]	8.23×10^7	$9.92 \pm 1.78 \times 10^6$	12.1 ± 2.16	0.02 ± 0.08
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Positive Control	9.07×10^7	$3.66 \pm 2.07 \times 10^6$	4.03 ± 2.28	-
Decontaminated	9.07×10^7	$1.83 \pm 1.65 \times 10^5$	0.20 ± 0.18	1.40± 0.39
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Glass				
Positive Control	9.20×10^7	$5.94 \pm 0.65 \times 10^7$	64.6 ± 7.04	-
Decontaminated	9.20×10^7	$1.81 \pm 2.80 \times 10^5$	0.20 ± 0.30	5.90 ± 3.0
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Decorative Laminate				
Positive Control	9.07×10^7	$2.10 \pm 0.84 \times 10^7$	23.2 ± 9.21	-
Decontaminated	9.07×10^7	$4.16 \pm 1.94 \times 10^6$	4.58 ± 2.14	0.75 ± 0.22
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Metal Ductwork				
Positive Control	9.20×10^7	$4.90 \pm 0.56 \times 10^7$	53.3 ± 6.06	-
Decontaminated	9.20×10^7	$0.87 \pm 1.38 \times 10^7$	9.46 ± 15.0	2.60 ± 2.8
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Wallboard Paper				
Positive Control	8.23×10^7	$2.56 \pm 0.71 \times 10^7$	31.1 ± 8.59	-
Decontaminated	8.23×10^7	$5.78 \pm 3.20 \times 10^6$	7.02 ± 3.88	0.74 ± 0.37
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Painted Concrete				
Positive Control	9.20×10^7	$2.51 \pm 1.03 \times 10^7$	27.2 ± 11.2	-
Decontaminated	9.20×10^7	$8.54 \pm 5.30 \times 10^6$	9.29 ± 5.77	0.54 ± 0.27
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated coupon
"-" Not Applicable

Table 4-5. Summary of Efficacy Values (Log Reduction) Obtained for pH-Amended Bleach[a]

Material	*B. anthracis* Ames	*B. anthracis* Sterne	*B. subtilis*	*G. stearothermophilus*
Industrial-Grade Carpet	0.28	**0.88**	0.19	0.02
Bare Wood	0.59	1.9	0.49	1.4
Glass	≥ 7.9	≥ 6.8	≥ 7.7	5.9
Decorative Laminate	≥ 7.2	≥ 6.6	6.9	**0.75**
Galvanized Metal Ductwork	≥ 7.7	≥ 7.4	≥ 7.8	**2.6**
Painted Wallboard Paper	2.0	**3.0**	**0.33**	**0.74**
Painted Concrete	1.0	**2.3**	**2.4**	0.54

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames

4.1.1.2 Qualitative Assessment of Residual Spores.

Based on previous decontamination studies,[3-6] it was anticipated that spores would not be completely recovered from coupons by the extraction process. Therefore, viable spores might remain on the test coupons following decontamination and extraction. As in previous decontamination studies, a qualitative assessment was performed to determine whether viable spores remained on the decontaminated and extracted test coupons; an assessment was also made to determine whether the decontaminated coupons with zero growth (zero growth indicated in Table 4-5 by "≥" values) in the quantitative measurement also showed no growth in the qualitative method.

To conduct the qualitative assessment, the test coupons from the quantitative assessment, following extraction, were transferred into tryptic soy broth culture medium and incubated for seven days at appropriate temperatures for growth. A cloudy liquid culture after incubation indicated that viable organisms of some type remained on the coupon after decontamination and extraction. For liquid cultures in which cloudiness was observed, a loop of the liquid sample was streaked onto a tryptic soy agar plate and incubated under appropriate conditions for growth. After incubation the plates were examined to determine qualitatively (morphologic comparison performed visually) if the observed growth was a pure culture of the organism that was inoculated onto the coupons, a mixture of the inoculated organism and other endogenous organisms, or a mixture of organisms, for example molds and bacteria. Because test coupons were not sterilized (only the coupon surface was wiped with 70% isopropanol) prior to inoculation, the presence of endogenous organisms was likely. Thus the indication of the presence of viable organisms (cloudy appearance in growth medium) did not necessarily indicate the presence of residual viable organisms that were spiked onto the test coupon. The percent of streak plates displaying only growth from the inoculated organism was 40, 58, 56, and 100% for *B. anthracis* Ames, *B. anthracis* Sterne, *B. subtilis*, and *G. stearothermophilus* spores, respectively.

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 4-6, 4-7, 4-8, and 4-9 for coupons spiked with *B. anthracis* Ames, *B. anthracis* Sterne, *B. subtilis*, and *G. stearothermophilus* spores, respectively. It should be noted that in several cases, growth was observed in blanks (for example see Table 4-

6, results for bare wood, Sample B1 for Day 1 and Day 7). This is due to growth of endogenous organisms.

Table 4-6. Liquid Culture Assessment of Coupons Inoculated with *Bacillus anthracis* Ames Spores following Extraction (pH-Amended Bleach)

Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Bare Wood														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Glass														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decorative Laminate														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Painted Concrete														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with *B. anthracis* Ames spores)
"+" = growth; "-" = no growth

Table 4-7. Liquid Culture Assessment of Coupons Inoculated with *Bacillus anthracis* Sterne Spores following Extraction (pH-Amended Bleach)

Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Inoculated, Not Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bare Wood														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Glass														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decorative Laminate														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Galvanized Metal Ductwork														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Painted Concrete														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with *B. anthracis* Sterne spores)
"+" = growth; "-" = no growth

Table 4-8. Liquid Culture Assessment of Coupons Inoculated with *Bacillus subtilis* Spores following Extraction (pH-Amended Bleach)

Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Inoculated, Not Decontaminated	-	-	+	+	-	+	-	-	-	+	+	-	+	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bare Wood														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glass														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decorative Laminate														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	-	+	+	-	-	-	-	-	+	+	-	-	-	-
Galvanized Metal Ductwork														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Painted Wallboard Paper														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Painted Concrete														
Inoculated, Not Decontaminated	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with *B. subtilis* spores)
"+" = growth; "-" = no growth

Table 4-9. Liquid Culture Assessment of Coupons Inoculated with *Geobacillus stearothermophilus* Spores following Extraction (pH-Amended Bleach)

Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Inoculated, Not Decontaminated	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Inoculated, Decontaminated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bare Wood														
Inoculated, Not Decontaminated	+	+	+	+	+	+	NR	+	+	+	+	+	+	NR
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Glass														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	+	+	-	+	-	+	-	+	+	-	+	-	+	-
Decorative Laminate														
Inoculated, Not Decontaminated	+	+	+	+	+	+	NR	+	+	+	+	+	+	NR
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Galvanized Metal Ductwork														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	-	-	-	-	+	+	-	-	-	-	-	+	+	-
Painted Wallboard Paper														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Painted Concrete														
Inoculated, Not Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Inoculated, Decontaminated	+	+	+	+	+	+	-	+	+	+	+	+	+	-

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with *G. stearothermophilus* spores)
NR = Not Recorded (data inadvertently not recorded)
"+" = growth; "-" = no growth

These results from the qualitative tests are generally consistent with the results from the quantitative tests (Table 4-10). Whereas in the quantitative tests, the amended bleach achieved high decontamination efficiency on hard, non-porous surfaces against *B. anthracis* Ames, *B. anthracis* Sterne, and *B. subtilis*, similar results were seen in the qualitative tests (i.e., few or no positive samples found). Also, whereas the quantitative tests shows notably less decontamination efficacy for amended bleach when used against *G. stearothermophilus* on hard surfaces, the qualitative tests showed all positive cultures for this spore type on hard surfaces. Finally, except for industrial grade carpet (due to its antimicrobial activity), amended bleach was not effective on porous surfaces for either the quantitative or qualitative tests for any spore type.

Table 4-10. Summary of Results Obtained from the Quantitative and Qualitative Assessments when Comparing Decontaminated Coupons (pH-Amended Bleach)

Material	*B. anthracis* Ames		*B. anthracis* Sterne		*B. subtilis*		*G. stearothermophilus*	
	A	B	A	B	A	B	A	B
Industrial-Grade Carpet	+	+	+	-	+	-	+	-
Bare Wood	+	+	+	+	+	+	+	+
Glass	-	-	-	-	-	-	+	c
Decorative Laminate	-	-	-	-	-*	c	+	+
Galvanized Metal Ductwork	-	-	-	-	-	-	+	c
Painted Wallboard Paper	+	+	+	+	+	+	+	+
Painted Concrete	+	+	+	+	+	+	+	+

A = Quantitative Assessment
B = Qualitative Assessment at seven days
"+" = observed CFU or growth; "-" = no observed CFU or no growth; "c" = combination of growth and no growth
* A small amount of growth was observed on only two of the seven replicates
Shading denotes inconsistent results

In the case of industrial-grade carpet, results were inconsistent. This is likely due to the susceptibility of vegetative growth to a broad-spectrum antibacterial compound (FlorSept®) in the carpet that leaches into the medium. It has been observed in previous testing (ref. 3-6) that FlorSept® (zinc omadine; also known as zinc pyrithione) appears to be both bactericidal and bacteriostatic in qualitative assessments. Apparently, the FlorSept® is not sporicidal, as it did not inactivate spores that were extracted and subsequently plated onto tryptic soy agar in this evaluation's quantitative assay. However, in the qualitative assessment, the carpet samples are incubated in the liquid broth for seven days. It is likely that during the seven day incubation the FlorSept® in the carpet leaches into the liquid broth, thereby asserting its bactericidal/bacteriostatic properties. In the past, we have demonstrated that if a sample of these "negative" liquid cultures is plated onto tryptic soy agar and incubated overnight that growth of the inoculated organism is observed. These results support the bactericidal/bacteriostatic properties of FlorSept®.

4.1.2 Damage to Coupons

Before and after decontamination of the test coupons, the decontaminated coupons were visually inspected; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were recorded. No damage (e.g., change in surface texture, color) or visible change was observed during this evaluation to any of the test coupons.

4.1.3 Other Factors

4.1.3.1 Operator Control

On each day of testing, the pH-amended bleach was prepared fresh by mixing household Clorox® bleach (5-6% sodium hypochlorite), 5% acetic acid, and distilled water. Titrations determined that in the pH-amended bleach solutions prepared, the mean total chlorine content was 6,215 ± 212 ppm and the pH was 6.81 ± 0.15. A NIST-traceable thermometer/hygrometer indicated that the temperature and RH were maintained in the test chamber within the specified range of 22 to 35°C and <70% RH.

4.1.3.2 Technology Spray Deposition

The pH-amended bleach was applied to glass and bare wood test coupons using a ten-second spray duration. The liquid was gravity-fed into an ultrasonic nozzle where it was mixed in an air stream at 40 psi to convert the liquid to droplets (fine mist, 10-50 microns in diameter for water). Gravimetric analysis was used to measure total spray deposition (as described in the test/QA plan[(1)]), which included the liquid adhering to the coupon surface as well as any collected run-off. This total mass was used to determine the total amount of spray deposition, or total mass of liquid contacting the surface of the coupon. For this assessment, separate pre-weighed coupons were sprayed simultaneously as two replicates of two coupons (N=4 total coupons). Following a ten-second spray, each coupon (plus collected runoff) was individually weighed and the mass determined. During each spray replicate, there was no observable difference in spray deposition (based on gravimetric results) between the two coupons. Total deposition was recorded at 0.32 ± 0.02 grams and 0.33 ± 0.02 grams of the pH-amended bleach onto glass and bare pine wood test coupons, respectively. Other spray times were used and the reproducibility of the spray application process is indicated by the data shown in Table 4-11. The high level of reproducibility ensures that the differences in efficacy are not likely due to differences in deposition of the decontamination technology.

4.1.3.3 Neutralization Methodology

Methods demonstration was performed to determine the neutralization efficiency against the pH-amended bleach using sodium thiosulfate. Neutralization of residual pH-amended bleach was necessary in order to obtain accurate decontamination efficacy data for the ten minute contact time. The neutralization results are shown in Table 4-12. The ten-second spray time resulted in approximately 0.325 mL of pH-amended bleach deposited on the coupon + collected runoff in a 50 mL conical tube, to which 10 mL of extraction buffer [phosphate-buffered saline (PBS) + 0.1% Triton X-100] containing sodium thiosulfate (STS) was added. It is known that the molar ratio for neutralization of hypochlorite with STS is 2:1; therefore, based upon the 0.325 mL of pH-amended bleach and 10 mL of extraction buffer, there were multiple variables that were calculated or measured so that neutralization efficiency could be determined. These variables included total chlorine concentration in the pH-amended bleach, total amount of pH-amended bleach deposited on the coupon + collected runoff for a ten second spray duration, molarity of pH-amended bleach, molarity of STS in the 10 mL of extraction buffer, and molar ratio of STS to hypochlorite. The results shown in Table 4-12 were based on a starting pH-amended bleach concentration of 6200 ppm. The target STS concentration for this 10 mL solution was calculated to be 0.086%. Higher (0.17%) and lower (0.043%) concentrations of STS were also tested to help demonstrate the most effective neutralization. STS can inhibit bacterial growth; therefore, the higher STS concentration was used to demonstrate the potential for any unreacted STS remaining in the extraction buffer to reduce neutralization efficiency by inactivating spores. The lower STS concentration was used to demonstrate the potential for any remaining non-neutralized bleach in the extraction buffer to potentially continue inactivating spores, thereby reducing neutralization efficiency. When compared to the controls (extraction buffer + spores with no STS), the calculated target STS concentration of 0.086% was the optimal STS concentration for neutralizing the bleach.

Table 4-11. Mean ± (SD) Weight of pH-Amended Bleach Deposited on Test Coupons (g)

Material[a]	Spray Time (seconds)[b]					Correlation
	1	5	10	15	20	Coefficient (R^2)
Glass	0.04 ± 0.002	0.17 ± 0.01	0.32 ± 0.02	0.60 ± 0.02	0.81 ± 0.08	0.9873
Bare Wood	0.04 ± 0.004	0.16 ± 0.01	0.33 ± 0.02	0.56 ± 0.08	0.76 ± 0.10	0.9946

[a] N = 4 coupons per time point
[b] Spray distance of 12 inches; spray pressure of 40 psi

Table 4-12. Neutralization Testing for pH-Amended Bleach

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
NaOCl + Spores[a]	9.80×10^7	0	0
NaOCl + PBS + Triton X-100 + Spores[ab]	9.80×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	9.80×10^7	8.59×10^7	-
NaOCl + PBS + Triton X-100 + 0.043% STS + Spores[ab]	9.80×10^7	4.30×10^7	50.1
NaOCl + PBS + Triton X-100 + 0.086% STS + Spores[ab]	9.80×10^7	8.45×10^7	98.3
NaOCl + PBS + Triton X-100 + 0.17% STS + Spores[ab]	9.80×10^7	5.62×10^7	65.4

[a] NaOCl volume corresponds to gravimetric deposition for ten-sec. spray duration
[b] Total volume is 10 mL
"-" Not Applicable

4.2 Ten Technologies Evaluated by Screening Test

Ten decontamination technologies were evaluated for decontamination efficacy against a biological warfare agent on one indoor surface. The technologies evaluated in the screening test are listed in Table 2-1. The evaluation followed the *Technology Testing and Evaluation Program Test/QA Plan for Evaluating Liquid and Foam Sporicidal Spray Decontaminants* (Version 1).[(1)]

Testing was undertaken as described in this technology evaluation report in order to rapidly screen sporicidal, spray-applied technologies for efficacy for inactivating spores on indoor materials. Prior to the screening test, a Vendor Agreement (including a Quick-Screen Protocol) was reviewed and signed by each vendor. The Quick-Screen Protocol specified that an automated spray apparatus would be used to apply the decontaminant and that spray application would be performed as specified by the vendor. Decontaminants were prepared daily in clean mixing chambers per vender instructions. Pre-established parameters – 12 inch spray distance, ten-second spray time, and 40 psi spray pressure would be used if vendors did not specify parameters. Some vendors expressed preference that their own spray equipment would be used and two vendors expressed concern that 40 psi might not be optimal for their technologies; however, none of the vendors recommended an alternate spray pressure. All of the ten technologies tested were sprayed using the same automated sprayer and the same spray-application conditions (12 inch spray distance, ten second spray time, and 40 psi spray pressure) in order to obtain comparative performance data. Also a contact time (contact time is the time that the spray-applied liquid was allowed to be in or on the test coupon prior to extraction and neutralization) following spraying was specified by the vendor (in the absence of specified spray time a ten minute contact time was used). The testing reported herein was performed in the Quick-Screen mode for the purpose of obtaining a preliminary assessment of the efficacy of different spray–applied technologies. Following this Quick-Screen, more testing was anticipated and performed for different types of technologies and for those that exhibited the greatest log reduction values.

Following the testing, the technology vendors were given the opportunity to review and comment on the draft results. Three of the four chlorine dioxide based technology vendors (ClorDiSys, Frontier Pharmaceutical, and BioProcess Associates) expressed concern that the spray system used in testing may not have been operated optimally for their product, and in particular, expressed concern with the high operating pressure. One vendor indicated that an air pressure of 40 psi would produce a fine mist (10 – 50 micron diameter droplet size). These relatively small size droplets, along with the air flow used to atomize the liquid, could lead to increased mass transfer of chlorine dioxide from the liquid to gas phase, thus potentially decreasing the chlorine dioxide concentration in the liquid and rendering it less effective. This phenomenon has not been verified.

Since the same test conditions were used for each technology, it is probable that the conditions for application would not be ideal for every technology. The data herein suggest that the method of application may impact efficacy. Therefore, based upon the results of this report, certain technologies (e.g., DioxiGuard, Exterm-6, and Selectrocide) should be further tested to evaluate various spray conditions as modification of these conditions may affect decontamination efficacy. We do note however that these technologies achieved complete inactivation of approximately 10^8 spores in the neutralization tests conducted (solution of spore inoculum + liquid decontamination technology); see Tables 4-18, 4-20, and 4-25.

The data also indicate that efficacy of spray applied technologies varies, depending upon the type of spores and the material on which or in which the spores reside. In addition, increasing the concentration of the decontaminant(s) (active ingredients) in a spray applied technology might lead to a marked improvement in the efficacy of the technology. In summary, the reader should view the data and results contained herein in the context of results of "Quick-Screening". More definitive testing may be needed in order to obtain data that fully characterize the sporicidal efficacy of each spray-applied technology.

Glass, a surface typically found inside an office building or a mass transit station, was used for this screening test. The test coupon surface measured 1.9 cm x 7.5 cm.

The biological agent used to evaluate the sporicidal decontamination technologies was *B. anthracis* Ames spores.

Four of the ten technologies that exhibited the greatest decontamination efficacy (Table 4-13) were selected for further evaluation. These four technologies were also selected because they represent four different types of sporicidal formulations that are available. Sections 4.3, 4.4, 4.5, and 4.6 detail the results of the further evaluation. The following sections summarize the results of the initial screening evaluations.

4.2.1 Decontamination Efficacy

4.2.1.1 Quantitative Assessment of the Log Reduction of Viable Organisms

Decontamination efficacy was calculated as the mean log reduction in viable organisms achieved by the decontamination technology. Decontamination efficacy was calculated as described in Section 4.1.1.1.

The decontamination efficacy of the ten sporicidal technologies for inactivating extractable, viable spores from the test materials ranged from 0.37 to ≥ 7.8 for *B. anthracis* Ames spores on glass coupons (Table 4-13) and varied statistically between technologies. Each of the ten technologies did reduce the number of viable spores that could be extracted from the glass coupons. No viable organisms were detected in any of the blank samples.

Table 4-13. Inactivation of *Bacillus anthracis* Ames Spores[a] on Glass (Ten Sporicidal Technologies)

Technology (Contact Time)	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
CASCAD SDF (30 minutes)				
Sprayed H_2O	9.67×10^7	$5.83 \pm 0.14 \times 10^7$	60.3 ± 1.5	-
Sprayed CASCAD SDF	9.67×10^7	$2.78 \pm 3.32 \times 10^2$	0	6.4 ± 1.6
Laboratory Blank[b]	0	0	0	-
Procedural Blank[c]	0	0	0	-
DeconGreen (30 minutes)				
Sprayed H_2O	9.67×10^7	$5.83 \pm 0.14 \times 10^7$	60.3 ± 1.5	-
Sprayed DeconGreen	9.67×10^7	$2.44 \pm 1.49 \times 10^4$	0.025 ± 0.015	3.4 ± 0.29
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
DioxiGuard (10 minutes)[d]				
Sprayed H_2O	9.40×10^7	$6.87 \pm 0.73 \times 10^7$	73.1 ± 7.7	-
Sprayed DioxiGuard	9.40×10^7	$4.04 \pm 1.35 \times 10^4$	0.04 ± 0.01	3.2 ± 0.13
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
EasyDecon 200 (60 minutes)				
Sprayed H_2O	9.77×10^7	$5.96 \pm 0.89 \times 10^7$	61.0 ± 9.1	-
Sprayed EasyDecon 200	9.77×10^7	$7.54 \pm 1.75 \times 10^6$	7.72 ± 1.80	0.91 ± 0.10
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Exterm-6 (60 minutes)[d]				
Sprayed H_2O	9.77×10^7	$5.96 \pm 0.89 \times 10^7$	61.0 ± 9.1	-
Sprayed Exterm-6	9.77×10^7	$4.65 \pm 2.47 \times 10^6$	4.76 ± 2.5	1.1 ± 0.20
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
HI-Clean 605 (90 minutes)				
Sprayed H_2O	9.60×10^7	$5.81 \pm 0.91 \times 10^7$	60.5 ± 9.5	-
Sprayed HI-Clean 605	9.60×10^7	0	0	≥7.8
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
HM-4100 (30 minutes)				
Sprayed H_2O	9.50×10^7	$5.93 \pm 0.74 \times 10^7$	62.4 ± 7.8	-
Sprayed HM-4100	9.50×10^7	$2.76 \pm 1.07 \times 10^7$	29.0 ± 11.2	0.37 ± 0.22
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
KlearWater (30 minutes)				
Sprayed H_2O	9.50×10^7	$5.93 \pm 0.74 \times 10^7$	62.4 ± 7.8	-
Sprayed KlearWater	9.50×10^7	0	0	≥7.8
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Peridox (10 minutes)				
Sprayed H_2O	9.40×10^7	$6.87 \pm 0.73 \times 10^7$	73.1 ± 7.7	-
Sprayed Peridox	9.40×10^7	0	0	≥7.8
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Selectrocide (10 minutes)[d]				
Sprayed H_2O	9.40×10^7	$6.87 \pm 0.73 \times 10^7$	73.1 ± 7.7	-
Sprayed Selectrocide	9.40×10^7	$3.87 \pm 0.71 \times 10^5$	0.41 ± 0.08	2.3 ± 0.08
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)

[b] Laboratory Blank = not inoculated, not decontaminated coupon

[c] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon

[d] Vendor expressed concern that the 40 psi spray pressure utilized for testing could potentially be too high for optimal performance of their product

"-" Not Applicable

4.2.2 Damage to Coupons

Before and after decontamination of the test coupons, the decontaminated coupons were visually inspected; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were recorded. No damage (e.g., change in surface texture, color) or visible change was observed during this evaluation to any of the test coupons.

4.2.3 Other Factors

4.2.3.1 Operator Control

On each day of testing, each of the ten sporicidal technologies was prepared fresh according to the vendor's instructions. Preparation procedures required simple procedures (dilution, stirring). The KlearWater technology did not require any preparation and was used as received.

A NIST-traceable thermometer/hygrometer indicated that the temperature and RH were maintained in the test chamber within the specified range of 22 to 35ºC and <70% RH.

4.2.3.2 Technology Spray Deposition

Each of the ten technologies was applied to the test coupons using a ten-second spray duration. Gravimetric analysis was performed on glass and bare pine wood coupons as described above in Section 4.1.3.2. The results of the gravimetric analysis for total spray deposition of each of the ten sporicidal technologies are shown in Table 4-14. For the four down-selected technologies, a daily verification of spray deposition was performed using a ten second spray duration and glass test coupons. This daily verification was performed to demonstrate consistency of the spray system with each decontaminant technology. This daily verification was performed to demonstrate consistency of the spray system with each decontaminant technology (p values calculated using t-test were all greater than 0.05, indicating essentially no difference in the values being compared).

Table 4-14. Spray Deposition of Water and Individual Technologies[ab]

Technology/Material[c]	Spray Deposition (mass in grams)
Water	
Glass	0.52 ± 0.03
Bare Pine Wood	0.52 ± 0.01
CASCAD SDF	
Glass	0.44 ± 0.01
Bare Pine Wood	0.43 ± 0.02
DeconGreen	
Glass	0.25 ± 0.01
Bare Pine Wood	0.26 ± 0.01
DioxiGuard	
Glass	0.42 ± 0.01
Bare Pine Wood	0.46 ± 0.03
EasyDecon 200	
Glass	0.24 ± 0.03
Bare Pine Wood	0.25 ± 0.01
Exterm-6	
Glass	0.44 ± 0.04
Bare Pine Wood	0.47 ± 0.02
HI-Clean 605	
Glass	0.48 ± 0.04
Bare Pine Wood	0.47 ± 0.04
HM-4100	
Glass	0.49 ± 0.06
Bare Pine Wood	0.44 ± 0.05
KlearWater	
Glass	0.45 ± 0.04
Bare Pine Wood	0.47 ± 0.02
Peridox	
Glass	0.43 ± 0.02
Bare Pine Wood	0.42 ± 0.02
Selectrocide	
Glass	0.51 ± 0.03
Bare Pine Wood	0.53 ± 0.02

[a] Data are expressed as mean (± SD)
[b] Spray distance of 12 inches; spray pressure of 40 psi; ten-second spray time
[c] N=4 coupons per time point

4.2.3.3 Neutralization Methodology

Methods demonstration was performed to determine the appropriate concentration of neutralizer for each technology. Neutralization was necessary in order to terminate the spore inactivating effect of the technology and obtain accurate efficacy data according to the vendor-specified contact time. The overall method used for testing neutralization efficiency for each technology is the same as used for pH-amended bleach (Section 4.1.3.3) and is described in the test/QA plan.[(1)] Details of the vendor-recommended neutralizer and selected concentration are shown in Table 4-15.

The neutralization results, using the neutralizer recommended by the vendors, are shown in Tables 4-16 to 4-25. A target concentration of neutralizer was calculated based upon vendor-

estimated concentration of decontaminant and spray-deposited mass of liquid following spraying. From this collected information, a range of neutralizer concentrations was evaluated for each technology. Six treatments (duration of each treatment was 15 minutes while shaking at 200 rpm) shown in Tables 4-16 to 4-25 were used to gather the data necessary to select a neutralization approach for each technology that was used in subsequent testing.

Table 4-15. Neutralizer for Each of Ten Commercially Available Technologies

Technology	Neutralizer (Vendor Recommended)	Final Concentration of Neutralizer [a]
CASCAD SDF	Sodium thiosulfate	0.5%
DeconGreen	Sodium thiosulfate	0.60%
DioxiGuard	Sodium thiosulfate	0.002%
EasyDecon 200	Sodium thiosulfate	0.5%
Exterm-6	Sodium thiosulfate	0.004%
HI-Clean 605	Sodium thiosulfate	0.08%
HM-4100	Sodium dodecylbenzene sulfonate	0.467 mL of 0.17%
KlearWater	Sodium thiosulfate	0.015%
Peridox	Dey/Engley broth + catalase	0.13 mL of Dey/Engley broth + 0.336 mL catalase
Selectrocide	Sodium thiosulfate	0.01%

[a] See Tables 4-16 through 4-25 for additional details

Table 4-16. Neutralization Testing for CASCAD SDF

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
CASCAD SDF + Spores[a]	4.80×10^7	0	0
CASCAD SDF + PBS + Triton X-100 + Spores[ab]	4.80×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	4.80×10^7	4.68×10^7	-
CASCAD SDF + PBS + Triton X-100 + 1.0% STS + Spores[ab]	4.80×10^7	4.07×10^7	87.0
CASCAD SDF + PBS + Triton X-100 + 0.5% STS + Spores[ab]	4.80×10^7	5.03×10^7	108
CASCAD SDF + PBS + Triton X-100 + 0.25% STS + Spores[ab]	4.80×10^7	0	0

[a] CASCAD volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-17. Neutralization Testing for DeconGreen

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
DeconGreen + Spores[a]	1.08×10^8	0	0
DeconGreen + PBS + Triton X-100 + Spores[ab]	1.08×10^8	TNTC	-
PBS + Triton X-100 + Spores (Control)[b]	1.08×10^8	9.88×10^7	-
DeconGreen + PBS + Triton X-100 + 0.30% STS + Spores[ab]	1.08×10^8	1.02×10^8	103
DeconGreen + PBS + Triton X-100 + 0.60% STS + Spores[ab]	1.08×10^8	9.46×10^7	95.7
DeconGreen + PBS + Triton X-100 + 1.20% STS + Spores[ab]	1.08×10^8	1.03×10^8	104

[a] DeconGreen volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
TNTC = Too Numerous to Count
"-" Not Applicable

Table 4-18. Neutralization Testing for DioxiGuard

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
DioxiGuard + Spores[a]	1.02×10^8	0	0
DioxiGuard + PBS + Triton X-100 + Spores[ab]	1.02×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.02×10^8	9.54×10^7	-
DioxiGuard + PBS + Triton X-100 + 0.02% STS+Spores[ab]	1.02×10^8	9.00×10^7	94.3
DioxiGuard + PBS + Triton X-100 + 0.002% STS+Spores[ab]	1.02×10^8	9.00×10^7	94.3
DioxiGuard + PBS + Triton X-100 + 0.0002% STS+Spores[ab]	1.02×10^8	3.48×10^4	0.0004

[a] DioxiGuard volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-19. Neutralization Testing for EasyDecon 200

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
EasyDecon 200 + Spores[a]	7.93×10^7	0	0
EasyDecon 200 + PBS + Triton X-100 + Spores[ab]	7.93×10^7	4.64×10^6	6.30
PBS + Triton X-100 + Spores (Control)[b]	7.93×10^7	7.36×10^7	-
EasyDecon 200 + PBS + Triton X-100 + 0.23% STS+ Spores[ab]	7.93×10^7	6.91×10^7	93.9
EasyDecon 200 + PBS + Triton X-100 + 0.50% STS + Spores[ab]	7.93×10^7	6.91×10^7	93.9
EasyDecon 200 + PBS + Triton X-100 + 1.0% STS + Spores[ab]	7.93×10^7	7.06×10^7	95.9

[a] EasyDecon 200volume [a]EasyDecon 200 volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-20. Neutralization Testing for Exterm-6

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
Exterm-6 + Spores[a]	8.43×10^7	0	0
Exterm-6 + PBS + Triton X-100 + Spores[ab]	8.43×10^7	0	0
PBS + Triton X-100 + Spores (Control)[b]	8.43×10^7	6.96×10^7	-
Exterm-6 + PBS + Triton X-100 + 0.04% STS + Spores[ab]	8.43×10^7	7.24×10^7	104
Exterm-6 + PBS + Triton X-100 + 0.004% STS + Spores[ab]	8.43×10^7	0	0
Exterm-6 + PBS + Triton X-100 + 0.0004% STS + Spores[ab]	8.43×10^7	0	0

[a] Exterm-6 volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-21. Neutralization Testing for HI-Clean 605

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
HI-Clean 605 + Spores[a]	4.77×10^8	0	0
HI-Clean 605 + PBS + Triton X-100 + Spores[ab]	4.77×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	4.77×10^8	3.10×10^8	-
HI-Clean 605 + PBS + Triton X-100 + 0.06% STS + Spores[ab]	4.77×10^8	2.68×10^8	86.5
HI-Clean 605 + PBS + Triton X-100 + 0.08% STS + Spores[ab]	4.77×10^8	2.96×10^8	95.5
HI-Clean 605 + PBS + Triton X-100 + 0.10% STS + Spores[ab]	4.77×10^8	1.67×10^8	53.8

[a] HI-Clean 605 volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-22. Neutralization Testing for HM-4100

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
HM-4100 + Spores[a]	1.00×10^8	1.07×10^7	10.5
HM-4100 + PBS + Triton X-100 + Spores[ab]	1.00×10^8	8.87×10^7	84.7
PBS + Triton X-100 + Spores (Control)[b]	1.00×10^8	1.02×10^8	-
HM-4100 + PBS + Triton X-100 + 1.0% SDS + Spores[ab]	1.00×10^8	8.19×10^7	80.3
HM-4100 + PBS + Triton X-100 + 0.047% SDS + Spores[ab]	1.00×10^8	9.84×10^7	96.5
HM-4100 + PBS + Triton X-100 + 0.023% SDS + Spores[ab]	1.00×10^8	7.82×10^7	76.7

[a] HM-4100 volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-23. Neutralization Testing for KlearWater

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
KlearWater + Spores[a]	1.05×10^8	0	0
KlearWater + PBS + Triton X-100 + Spores[ab]	1.05×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.05×10^8	9.99×10^7	-
KlearWater + PBS + Triton X-100 + 0.005% STS + Spores[ab]	1.05×10^8	9.12×10^7	91.3
KlearWater + PBS + Triton X-100 + 0.015% STS + Spores[ab]	1.05×10^8	9.69×10^7	97.2
KlearWater + PBS + Triton X-100 + 0.03% STS + Spores[ab]	1.05×10^8	9.59×10^7	95.9

[a] KlearWater volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-24. Neutralization Testing for Peridox

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
Peridox + Spores[a]	1.01×10^8	0	0
Peridox + PBS + Triton X-100 + Spores[ab]	1.01×10^8	7.64×10^7	82.9
PBS + Triton X-100 + Spores (Control)[b]	1.01×10^8	9.22×10^7	-
Peridox + PBS + Triton X-100 + 0.3mL DE/CAT+Spores[ab]	1.01×10^8	8.44×10^7	91.5
Peridox + PBS + Triton X-100 +0.426mL DE/CAT+Spores[ab]	1.01×10^8	1.01×10^8	110
Peridox + PBS + Triton X-100 + 0.5mL DE/CAT+Spores[ab]	1.01×10^8	8.10×10^7	87.8

[a] Peridox volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

Table 4-25. Neutralization Testing for Selectrocide

Treatment	Inoculum (CFU)	Total Observed CFU	% of Control
Selectrocide + Spores[a]	1.00×10^8	0	0
Selectrocide + PBS + Triton X-100 + Spores[ab]	1.00×10^8	0	0
PBS + Triton X-100 + Spores (Control)[b]	1.00×10^8	1.02×10^8	-
Selectrocide + PBS + Triton X-100 + 0.02% STS + Spores[ab]	1.00×10^8	9.90×10^7	96.9
Selectrocide + PBS + Triton X-100 + 0.01% STS + Spores[ab]	1.00×10^8	1.03×10^8	101
Selectrocide + PBS + Triton X-100 + 0.005% STS +Spores[ab]	1.00×10^8	9.88×10^7	96.8

[a] Selectrocide volume corresponds to spray deposited mass shown in Table 4-14
[b] Total volume is 10 mL (PBS + Triton X-100) plus volume (for mass to volume conversion, 1 g = 1 mL) of corresponding spray deposited mass shown in Table 4-14)
"-" Not Applicable

4.3 CASCAD Surface Decontamination Foam

CASCAD Surface Decontamination Foam (SDF), one of the four down-selected technologies, was evaluated for decontamination efficacy against a biological warfare agent and two additional organisms on three indoor surfaces. Various structural, decorative, and functional surfaces typically found inside an office building or a mass transit station were used to evaluate the sporicidal decontamination technology. In the case of this further testing, three of the seven coupon types were employed. The test surfaces (coupons measuring 1.9 cm x 7.5 cm) are listed below:

- Industrial-grade carpet
- Bare wood (pine lumber)
- Galvanized metal ductwork.

The biological agent used to evaluate the sporicidal decontamination technology was *B. anthracis* Ames spores. To provide comparisons with the *B. anthracis* Ames results, the organisms, *B. subtilis* (ATCC 19659) and *G. stearothermophilus* (ATCC 12980) were used. The following sections summarize the results of these evaluations.

4.3.1 Decontamination Efficacy

4.3.1.1 Quantitative Assessment of the Log Reduction of Viable Organisms

Decontamination efficacy was calculated as the mean log reduction in viable organisms achieved by the decontamination technology. Decontamination efficacy was calculated as defined in Section 4.1.1.1.

The decontamination efficacy of CASCAD SDF for inactivating extractable, viable spores from the test materials ranged from 0.58 to 5.52 for *B. anthracis* Ames spores for all three test materials (Table 4-26). The decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.78 to 4.49 and 0.83 to 4.61, respectively (Tables 4-27 and 4-28). No viable organisms were detected in any of the blank samples. The decontamination efficacy results for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* are summarized in Table 4-29. The decontamination efficacy varied with respect to both the agent or organism and the test material.

Statistically significant differences in efficacy for *B. subtilis* and *G. stearothermophilus* compared to *B. anthracis* Ames were observed for two of the three test materials (Table 4-29). For bare wood, the decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spores were statistically greater than that of *B. anthracis* Ames. However, a statistically lower decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spore compared to *B. anthracis* Ames spores was observed on galvanized metal.

Table 4-26. Inactivation of *Bacillus anthracis* Ames Spores[a] (CASCAD SDF)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	9.60×10^7	$4.20 \pm 0.82 \times 10^7$	43.8 ± 8.50	-
Sprayed CASCAD SDF[c]	9.60×10^7	$3.98 \pm 0.96 \times 10^6$	4.15 ± 1.00	1.03 ± 0.10
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	9.60×10^7	$6.95 \pm 1.24 \times 10^6$	7.24 ± 1.30	-
Sprayed CASCAD SDF	9.60×10^7	$1.83 \pm 0.13 \times 10^6$	1.91 ± 0.14	0.58 ± 0.03
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	9.60×10^7	$5.91 \pm 0.53 \times 10^7$	61.6 ± 5.56	-
Sprayed CASCAD SDF	9.60×10^7	$2.61 \pm 3.01 \times 10^2$	<0.001	5.52 ± 0.37
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction), "-" Not Applicable
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon

Table 4-27. Inactivation of *Bacillus subtilis* Spores[a] (CASCAD SDF)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.06×10^8	$4.27 \pm 0.54 \times 10^7$	40.3 ± 5.13	-
Sprayed CASCAD SDF[c]	1.06×10^8	$4.87 \pm 0.84 \times 10^6$	4.60 ± 0.79	0.95 ± 0.08
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.06×10^8	$4.97 \pm 0.66 \times 10^6$	4.69 ± 0.63	-
Sprayed CASCAD SDF	1.06×10^8	$8.24 \pm 1.19 \times 10^5$	0.78 ± 0.11	0.78 ± 0.06
Laboratory Blank		0	0	-
Procedural Blank		0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.06×10^8	$6.87 \pm 0.82 \times 10^7$	64.8 ± 7.77	-
Sprayed CASCAD SDF	1.06×10^8	$2.34 \pm 0.84 \times 10^3$	<0.01	4.49 ± 0.16
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction), "-" Not Applicable
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon

Table 4-28. Inactivation of *Geobacillus stearothermophilus* Spores[a] (CASCAD SDF)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.02×10^8	$4.71 \pm 0.36 \times 10^7$	46.2 ± 3.57	-
Sprayed CASCAD SDF[c]	1.02×10^8	$4.98 \pm 1.42 \times 10^6$	4.88 ± 1.40	0.99 ± 0.14
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.02×10^8	$3.26 \pm 0.21 \times 10^6$	3.20 ± 0.20	-
Sprayed CASCAD SDF	1.02×10^8	$4.98 \pm 1.45 \times 10^5$	0.49 ± 0.14	0.83 ± 0.14
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.02×10^8	$7.84 \pm 0.38 \times 10^7$	76.9 ± 3.73	-
Sprayed CASCAD SDF	1.02×10^8	$2.09 \pm 0.84 \times 10^3$	<0.01	4.61 ± 0.19
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-29. Summary of Efficacy Values Obtained for CASCAD SDF[a]

Material	*B. anthracis* Ames	*B. subtilis*	*G. stearothermophilus*
Industrial-Grade Carpet	1.03	0.95	0.99
Bare Wood	0.58	**0.78**	**0.83**
Galvanized Metal Ductwork	5.52	**4.49**	**4.61**

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames

4.3.1.2 Qualitative Assessment of Residual Spores

Based on previous decontamination studies,[3-6] it was anticipated that spores would not be completely recovered from coupons by the extraction process. Therefore, viable spores might remain on the test coupons. As in previous decontamination studies, a qualitative assessment was performed, as detailed in Section 4.1.1.2, to determine whether viable spores remained on the decontaminated and extracted test coupons.

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 4-30, 4-31, and 4-32 for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*, respectively. In all cases viable spores remained on the coupons. Streak plates displayed only growth from the inoculated organism. This growth in which only the inoculated organism was observed on the streak plates reflects the improved procedure for sterilizing the coupons [i.e., gamma irradiation (40 KiloGray) of coupons] prior to testing.

Table 4-30. Liquid Culture Assessment of Coupons Inoculated with *Bacillus anthracis* Ames Spores following Extraction (CASCAD SDF)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	-	-	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-31. Liquid Culture Assessment of Coupons Inoculated with *Bacillus subtilis* Spores following Extraction (CASCAD SDF)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	-	+	+	+	-	-	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-32. Liquid Culture Assessment of Coupons Inoculated with *Geobacillus stearothermophilus* Spores following Extraction (CASCAD SDF)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed CASCAD SDF	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

The results of the decontaminated coupons from the qualitative assessment were consistent with the results from the quantitative assessment and are summarized in Table 4-33. In all cases where growth of spores was observed in the quantitative method, there was a corresponding observation of growth in the liquid culture medium after seven days incubation. In all cases, viable spores were shown to be present in the positive controls using both the quantitative and qualitative methods.

Table 4-33. Summary of Results Obtained from the Quantitative and Qualitative Assessments when Comparing Decontaminated Coupons (CASCAD SDF)

Material	*B. anthracis* Ames		*B. subtilis*		*G. stearothermophilus*	
	A	B	A	B	A	B
Industrial-Grade Carpet	+	+	+	+	+	+
Bare Wood	+	+	+	+	+	+
Galvanized Metal Ductwork	+	+	+	+	+	+

A = Quantitative Assessment
B = Qualitative Assessment at seven days
"+" = observed CFU or growth; "-" = no observed CFU or no growth

4.3.2 Damage to Coupons

Before and after decontamination of the test coupons, the decontaminated coupons were visually inspected; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were recorded. No damage (e.g., change in surface texture, color) or visible change was observed during this evaluation to any of the test coupons.

4.3.3 Other Factors

4.3.3.1 Operator Control

On each day of testing, the sporicidal technology was prepared fresh according to the vendor's instructions. A NIST-traceable thermometer/hygrometer indicated that the temperature and RH were maintained within the specified range of 22 to 35ºC and <70% RH.

4.3.3.2 Technology Spray Deposition

Gravimetric analysis for total deposition (coupon + run-off) for the sporicidal technology and daily verification of spray performance were performed. The results of the initial CASCAD SDF spray deposition are shown in Table 4-14 for glass and bare pine wood. Daily verification of the amount of spray deposition based on three separate days (N=4 glass coupons per day) resulted in a mean (± SD) mass of 0.45 ± 0.01 grams, which was not statistically different (p = 0.559) than the initial spray deposition on glass coupons of 0.44 ± 0.01 grams.

4.3.3.3 Neutralization Methodology

The approach used for testing neutralization efficiency for CASCAD SDF is described in Section 4.2.3.3 and is also described in the test/QA plan.[1] Details of the vendor-recommended neutralizer and selected concentration are shown in Table 4-15.

4.4 HI-Clean 605

HI-Clean 605 was evaluated for decontamination efficacy as described in Section 4.3. The following sections summarize the results of these evaluations.

4.4.1 Decontamination Efficacy

4.4.1.1 Quantitative Assessment of the Log Reduction of Viable Organisms

Decontamination efficacy was calculated as the mean log reduction in viable organisms achieved by the decontamination technology. Decontamination efficacy was calculated as defined in Section 4.1.1.1.

The decontamination efficacy of HI-Clean 605 for inactivating extractable, viable spores from the test materials ranged from 0.92 to 4.27 for *B. anthracis* Ames spores for all three test materials (Table 4-34). The decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.02 to 2.46 and 0.47 to 1.31, respectively (Tables 4-35 and 4-36). No viable organisms were detected in any of the blank samples. The summary of decontamination efficacy results for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* are summarized in Table 4-37. The decontamination efficacy varied with respect to both the agent or organism and the test material.

Statistically significant differences in efficacy compared to *B. anthracis* were observed for two of the three test materials (Table 4-37). For bare wood and galvanized metal, the decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spores were statistically lower than that of *B. anthracis* Ames.

Table 4-34. Inactivation of *Bacillus anthracis* Ames Spores[a] (HI-Clean 605)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	9.17×10^7	$5.65 \pm 0.70 \times 10^7$	61.7 ± 7.59	-
Sprayed HI-Clean 605[c]	9.17×10^7	$1.40 \pm 1.23 \times 10^6$	1.53 ± 1.34	1.70 ± 0.28
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	9.17×10^7	$5.91 \pm 0.92 \times 10^6$	6.45 ± 1.01	-
Sprayed HI-Clean 605	9.17×10^7	$7.21 \pm 1.67 \times 10^5$	0.79 ± 0.18	0.92 ± 0.11
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	9.17×10^7	$6.81 \pm 0.93 \times 10^7$	74.3 ± 10.2	-
Sprayed HI-Clean 605	9.17×10^7	$3.73 \pm 0.96 \times 10^3$	0.0041 ± 0.001	4.27 ± 0.11
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-35. Inactivation of *Bacillus subtilis* Spores[a] (HI-Clean 605)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.03×10^8	$2.17 \pm 1.18 \times 10^7$	21.1 ± 11.5	-
Sprayed HI-Clean 605[c]	1.03×10^8	$1.05 \pm 0.93 \times 10^6$	1.02 ± 0.90	1.42 ± 0.30
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.03×10^8	$3.76 \pm 1.33 \times 10^6$	3.65 ± 1.29	-
Sprayed HI-Clean 605	1.03×10^8	$3.59 \pm 0.55 \times 10^6$	3.48 ± 0.53	0.02 ± 0.07
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.03×10^8	$7.13 \pm 1.04 \times 10^7$	69.2 ± 10.1	-
Sprayed HI-Clean 605	1.03×10^8	$2.87 \pm 1.47 \times 10^5$	0.28 ± 0.14	2.46 ± 0.28
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-36. Inactivation of *Geobacillus stearothermophilus* Spores[a] (HI-Clean 605)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.09×10^8	$1.76 \pm 1.53 \times 10^7$	16.2 ± 14.0	-
Sprayed HI-Clean 605[c]	1.09×10^8	$1.14 \pm 1.06 \times 10^6$	1.04 ± 0.97	1.31 ± 0.33
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.09×10^8	$1.54 \pm 1.14 \times 10^7$	14.1 ± 10.5	-
Sprayed HI-Clean 605	1.09×10^8	$6.17 \pm 2.90 \times 10^6$	5.66 ± 2.66	0.47 ± 0.32
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.09×10^8	$9.04 \pm 1.08 \times 10^7$	82.9 ± 9.87	-
Sprayed HI-Clean 605	1.09×10^8	$6.95 \pm 3.42 \times 10^6$	6.38 ± 3.14	1.15 ± 0.20
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-37. Summary of Efficacy Values Obtained for HI-Clean 605[a]

Material	*B. anthracis* Ames	*B. subtilis*	*G. stearothermophilus*
Industrial-Grade Carpet	1.70	1.42	1.31
Bare Wood	0.92	**0.02**	**0.47**
Galvanized Metal Ductwork	4.27	**2.46**	**1.15**

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames

4.4.1.2 Qualitative Assessment of Residual Spores

Based on previous decontamination studies,[3-6] it was anticipated that spores would not be completely recovered from coupons by the extraction process. Therefore, viable spores might remain on the test coupons. As in previous decontamination studies, a qualitative assessment was performed, as detailed in Section 4.1.1.2, to determine whether viable spores remained on the decontaminated and extracted test coupons.

Results from the liquid culture growth assessment of coupons as one and seven days post-decontamination are provided in Tables 4-38, 4-39, and 4-40 for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*, respectively. In all cases viable spores remained on the coupons. Streak plates displayed only growth from the inoculated organism. This growth in which only the inoculated organism was observed on the streak plates reflects the improved procedures for sterilizing the coupons prior to testing.

Table 4-38. Liquid Culture Assessment of Coupons Inoculated with *Bacillus anthracis* Ames Spores following Extraction (HI-Clean 605)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-39. Liquid Culture Assessment of Coupons Inoculated with *Bacillus subtilis* Spores following Extraction (HI-Clean 605)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-40. Liquid Culture Assessment of Coupons Inoculated with *Geobacillus stearothermophilus* Spores following Extraction (HI-Clean 605)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed HI-Clean 605	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

The results of the decontaminated coupons from the qualitative assessment were consistent with the results from the quantitative assessment and are summarized in Table 4-41. In all cases where growth of spores was observed in the quantitative method, there was a corresponding observation of growth in the liquid culture medium after seven days incubation. In all cases, viable spores were shown to be present in the positive controls using both the quantitative and qualitative methods.

Table 4-41. Summary of Results Obtained from the Quantitative and Qualitative Assessments when Comparing Decontaminated Coupons (HI-Clean 605)

Material	*B. anthracis* Ames		*B. subtilis*		*G. stearothermophilus*	
	A	B	A	B	A	B
Industrial-Grade Carpet	+	+	+	+	+	+
Bare Wood	+	+	+	+	+	+
Galvanized Metal Ductwork	+	+	+	+	+	+

A = Quantitative Assessment
B = Qualitative Assessment at seven days
"+" = observed CFU or growth; "-" = no observed CFU or no growth

4.4.2 Damage to Coupons

Before and after decontamination of the test coupons, the decontaminated coupons were visually inspected; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were recorded. No damage (e.g., change in surface texture, color) or visible change was observed during this evaluation to any of the test coupons.

4.4.3 Other Factors

4.4.3.1 Operator Control

On each day of testing, the sporicidal technology was prepared fresh according to the vendor's instructions.

A NIST-traceable thermometer/hygrometer indicated that the temperature and RH were maintained within the specified range of 22 to 35ºC and <70% RH.

4.4.3.2 Technology Spray Deposition

Gravimetric analysis for total deposition (coupon + run-off) for the sporicidal technology and daily verification of deposition were performed and are detailed in Section 4.2.3.2. The results of the initial HI-Clean 605 spray deposition are shown in Table 4-14 for glass and bare pine wood. Daily verification of the amount of spray deposition based on three separate days (N=4 glass coupons per day) resulted in a mean (± SD) mass of 0.47 ± 0.02 grams, which was not statistically different (p = 0.809) than the initial spray deposition on glass coupons of 0.48 ± 0.04 grams.

4.4.3.3 Neutralization Methodology

The approach used for testing neutralization efficiency for HI-Clean 605 is described in Section 4.2.3.3 and is also described in the test/QA plan.[1] Details of the vendor-recommended neutralizer and selected concentration are shown in Table 4-15.

4.5 KlearWater

KlearWater was evaluated for decontamination efficacy as described in Section 4.3. The following sections summarize the results of these evaluations.

4.5.1 Decontamination Efficacy

4.5.1.1 Quantitative Assessment of the Log Reduction of Viable Organisms

Decontamination efficacy was calculated as the log reduction in viable organisms achieved by the decontamination technology. Efficacy was calculated as defined in Section 4.1.1.1.

The decontamination efficacy of KlearWater for inactivating extractable, viable spores from the test materials ranged from 0.05 to 0.92 for *B. anthracis* Ames spores for all three test materials (Table 4-42). The decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.12 to 0.30 and 0.72 to 0.98, respectively (Tables 4-43 and 4-44). No viable organisms were detected in any of the blank samples. The summary of decontamination efficacy results for *B. anthracis* Ames, *B. subtilis,* and *G. stearothermophilus* are summarized in Table 4-45. The decontamination efficacy varied with respect to both the agent or organism and the test material.

Statistically significant differences in efficacy compared to *B. anthracis* Ames were observed for all three test materials (Table 4-45). For industrial carpet, the decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spores was statistically greater than that of *B. anthracis* Ames. For bare wood, the decontamination efficacy for *G. stearothermophilus* spores was statistically greater than that of *B. anthracis* Ames. However, statistically lower decontamination efficacy for *B. subtilis* spores was observed on galvanized metal when compared to *B. anthracis* Ames.

Table 4-42. Inactivation of *Bacillus anthracis* Ames Spores[a] (KlearWater)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	9.53×10^7	$5.29 \pm 0.82 \times 10^7$	55.5 ± 8.58	-
Sprayed KlearWater[c]	9.53×10^7	$4.78 \pm 1.00 \times 10^7$	50.1 ± 10.5	0.05 ± 0.09
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	9.53×10^7	$7.76 \pm 1.01 \times 10^6$	8.14 ± 1.06	-
Sprayed KlearWater	9.53×10^7	$4.61 \pm 1.43 \times 10^6$	4.84 ± 1.50	0.24 ± 0.13
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	9.53×10^7	$5.02 \pm 1.50 \times 10^7$	52.7 ± 15.7	-
Sprayed KlearWater	9.53×10^7	$6.08 \pm 1.07 \times 10^6$	6.38 ± 1.12	0.92 ± 0.07
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-43. Inactivation of *Bacillus subtilis* Spores[a] (KlearWater)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.08×10^8	$2.67 \pm 0.79 \times 10^7$	24.8 ± 7.31	-
Sprayed KlearWater[c]	1.08×10^8	$1.35 \pm 0.16 \times 10^7$	12.5 ± 1.48	0.30 ± 0.05
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.08×10^8	$3.62 \pm 0.85 \times 10^6$	3.35 ± 0.79	-
Sprayed KlearWater	1.08×10^8	$2.82 \pm 0.58 \times 10^6$	2.61 ± 0.53	0.12 ± 0.10
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.08×10^8	$4.60 \pm 0.60 \times 10^7$	42.6 ± 5.56	-
Sprayed KlearWater	1.08×10^8	$3.25 \pm 0.22 \times 10^7$	30.1 ± 2.06	0.15 ± 0.03
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-44. Inactivation of *Geobacillus stearothermophilus* Spores[a] (KlearWater)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.05×10^8	$2.47 \pm 1.70 \times 10^7$	23.5 ± 16.2	-
Sprayed KlearWater[c]	1.05×10^8	$4.89 \pm 1.45 \times 10^6$	4.66 ± 1.38	0.72 ± 0.13
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.05×10^8	$3.46 \pm 0.45 \times 10^6$	3.29 ± 0.43	-
Sprayed KlearWater	1.05×10^8	$3.69 \pm 0.69 \times 10^5$	0.35 ± 0.07	0.98 ± 0.08
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.05×10^8	$7.84 \pm 0.48 \times 10^7$	74.7 ± 4.61	-
Sprayed KlearWater	1.05×10^8	$8.25 \pm 0.66 \times 10^6$	7.85 ± 0.63	0.98 ± 0.04
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-45. Summary of Efficacy Values Obtained for KlearWater[a]

Material	*B. anthracis* Ames	*B. subtilis*	*G. stearothermophilus*
Industrial-Grade Carpet	0.05	**0.30**	**0.72**
Bare Wood	0.24	0.12	**0.98**
Galvanized Metal Ductwork	0.92	**0.15**	0.98

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames

4.5.1.2 Qualitative Assessment of Residual Spores

Based on previous decontamination studies,[3-6] it was anticipated that spores would not be completely recovered from coupons by the extraction process. Therefore, viable spores might remain on the test coupons. As in previous decontamination studies, a qualitative assessment was performed, as detailed in Section 4.1.1.2, to determine whether viable spores remained on the decontaminated and extracted test coupons

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 4-46, 4-47, and 4-48 for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*, respectively. Streak plates displayed only growth from the inoculated organism. This growth in which only the inoculated organism was observed on the streak plates reflects the improved procedures for sterilizing the coupons prior to testing.

Table 4-46. Liquid Culture Assessment of Coupons Inoculated with *Bacillus anthracis* Ames Spores following Extraction (KlearWater)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	-	+	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-47. Liquid Culture Assessment of Coupons Inoculated with *Bacillus subtilis* Spores following Extraction (KlearWater)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-48. Liquid Culture Assessment of Coupons Inoculated with *Geobacillus stearothermophilus* Spores following Extraction (KlearWater)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed KlearWater	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

The results of the decontaminated coupons from the qualitative assessment were consistent with the results from the quantitative assessment and are summarized in Table 4-49. In all cases where growth of spores was observed in the quantitative method, there was a corresponding observation of growth in the liquid culture medium after seven days incubation. In all cases, viable spores were shown to be present in the positive controls using both the quantitative and qualitative methods.

Table 4-49. Summary of Results Obtained from the Quantitative and Qualitative Assessments when Comparing Decontaminated Coupons (KlearWater)

Material	***B. anthracis* Ames**		***B. subtilis***		***G. stearothermophilus***	
	A	B	A	B	A	B
Industrial-Grade Carpet	+	+	+	+	+	+
Bare Wood	+	+	+	+	+	+
Galvanized Metal Ductwork	+	+	+	+	+	+

A = Quantitative Assessment
B = Qualitative Assessment at seven days
"+" = observed CFU or growth; "-" = no observed CFU or no growth

4.5.2 Damage to Coupons

Before and after decontamination of the test coupons, the decontaminated coupons were visually inspected; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were recorded. No damage (e.g., change in surface texture, color) or visible change was observed during this evaluation to any of the test coupons.

4.5.3 Other Factors

4.5.3.1 Operator Control

The KlearWater technology did not require any preparation and was used as received. A NIST-traceable thermometer/hygrometer indicated that the temperature and RH were maintained within the specified range of 22 to 35ºC and <70% RH.

4.5.3.2 Technology Spray Deposition

Gravimetric analysis for total deposition (coupon + run-off) for the sporicidal technology and daily verification of deposition were performed and are detailed in Section 4.2.3.2. The results of the initial KlearWater spray deposition are shown in Table 4-14 for glass and bare pine wood. Daily verification of the amount of spray deposition based on three separate days (N=4 glass coupons per day) resulted in a mean (± SD) mass of 0.46 ± 0.01 grams, which was not statistically different (p = 0.526) than the initial spray deposition on glass coupons of 0.45 ± 0.04 grams.

4.5.3.3 Neutralization Methodology

The approach used for testing neutralization efficiency for KlearWater is described in Section 4.2.3.3 and is also described in the test/QA plan.[(1)] Details of the vendor-recommended neutralizer and selected concentration are shown in Table 4-15.

4.6 Peridox

Peridox was evaluated for decontamination efficacy as described in Section 4.3. The following sections summarize the results of these evaluations.

4.6.1 Decontamination Efficacy

4.6.1.1 Quantitative Assessment of the Log Reduction of Viable Organisms

Decontamination efficacy was calculated as the mean log reduction in viable organisms achieved by the decontamination technology. Decontamination efficacy was calculated as defined in Section 4.1.1.1.

The decontamination efficacy of Peridox for inactivating extractable, viable spores from the test materials ranged from 0.87 to 1.05 for *B. anthracis* Ames spores for all three test materials (Table 4-50). The decontamination efficacy for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.72 to 2.08 and 1.93 to 4.38, respectively (Tables 4-51 and 4-52). No viable organisms were detected in any of the blank samples. The summary of decontamination efficacy results for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus* are summarized in Table 4-53. The decontamination efficacy varied with respect to both the agent or organism and the test material.

Statistically significant differences in efficacy compared to *B. anthracis* Ames were observed for all three test materials (Table 4-53). For industrial carpet and galvanized metal, the decontamination efficacy for *B. subtilis* spores was statistically greater than that of *B. anthracis* Ames. Also, the decontamination efficacy was statistically greater for *G. stearothermophilus* spores when compared to *B. anthracis* Ames spores for all three test materials – industrial carpet, bare wood, and galvanized metal.

Table 4-50. Inactivation of *Bacillus anthracis* Ames Spores[a] (Peridox)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	9.83×10^7	$4.45 \pm 0.70 \times 10^7$	45.2 ± 7.15	-
Sprayed Peridox[c]	9.83×10^7	$6.03 \pm 0.66 \times 10^6$	6.13 ± 0.67	0.87 ± 0.05
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	9.83×10^7	$5.90 \pm 1.06 \times 10^6$	6.00 ± 1.08	-
Sprayed Peridox	9.83×10^7	$5.46 \pm 1.82 \times 10^5$	0.56 ± 0.18	1.05 ± 0.15
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	9.83×10^7	$6.18 \pm 0.67 \times 10^7$	62.9 ± 6.77	-
Sprayed Peridox	9.83×10^7	$6.07 \pm 1.42 \times 10^6$	6.17 ± 1.45	1.02 ± 0.10
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-51. Inactivation of *Bacillus subtilis* Spores[a] (Peridox)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.05×10^8	$4.22 \pm 0.33 \times 10^7$	40.2 ± 3.17	-
Sprayed Peridox[c]	1.05×10^8	$6.19 \pm 2.45 \times 10^5$	0.59 ± 0.23	1.86 ± 0.15
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.05×10^8	$3.95 \pm 0.74 \times 10^6$	3.76 ± 0.70	-
Sprayed Peridox	1.05×10^8	$7.97 \pm 2.97 \times 10^5$	0.76 ± 0.28	0.72 ± 0.17
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.05×10^8	$5.06 \pm 0.62 \times 10^7$	48.2 ± 5.91	-
Sprayed Peridox	1.05×10^8	$6.48 \pm 3.98 \times 10^5$	0.62 ± 0.38	2.08 ± 0.58
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-52. Inactivation of *Geobacillus stearothermophilus* Spores[a] (Peridox)

Test Material	Inoculum (CFU)	Total Observed CFU	% Recovery	Decontamination Efficacy
Industrial-Grade Carpet				
Sprayed H_2O[b]	1.03×10^8	$5.88 \pm 1.09 \times 10^7$	57.1 ± 10.6	-
Sprayed Peridox[c]	1.03×10^8	$3.30 \pm 2.03 \times 10^3$	<0.01	4.38 ± 0.45
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Bare Wood				
Sprayed H_2O	1.03×10^8	$3.46 \pm 0.51 \times 10^6$	3.36 ± 0.50	-
Sprayed Peridox	1.03×10^8	$9.03 \pm 7.86 \times 10^2$	<0.01	3.72 ± 0.37
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Galvanized Ductwork				
Sprayed H_2O	1.03×10^8	$7.62 \pm 0.32 \times 10^7$	73.9 ± 3.09	-
Sprayed Peridox	1.03×10^8	$9.71 \pm 4.23 \times 10^5$	0.94 ± 0.41	1.93 ± 0.20
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as mean (± SD) total number of spores (CFU) observed, percent recovery, and decontamination efficacy (log reduction)
[b] Inoculated, not decontaminated coupon
[c] Inoculated, decontaminated coupon
[d] Laboratory Blank = not inoculated, not decontaminated coupon
[e] Procedural Blank = not inoculated, decontaminated (H_2O-sprayed) coupon
"-" Not Applicable

Table 4-53. Summary of Efficacy Values Obtained for Peridox[a]

Material	*B. anthracis* Ames	*B. subtilis*	*G. stearothermophilus*
Industrial-Grade Carpet	0.87	**1.86**	**4.38**
Bare Wood	1.05	0.72	**3.72**
Galvanized Metal Ductwork	1.02	**2.08**	**1.93**

[a] Numbers in bold are statistically different ($p \leq 0.05$) from *B. anthracis* Ames

4.6.1.2 Qualitative Assessment of Residual Spores

Based on previous decontamination studies, [3-6] it was anticipated that spores would not be completely recovered from coupons by the extraction process. Therefore, viable spores might remain on the test coupons. As in previous decontamination studies, a qualitative assessment was performed, as detailed in Section 4.1.1.2, to determine whether viable spores remained on the decontaminated and extracted test coupons.

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Tables 4-54, 4-55, and 4-56 for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*, respectively. In all cases viable *G. stearothermophilus* spores remained on the coupons after seven days. Streak plates displayed only growth from the inoculated organism. This growth in which only the inoculated organism was observed on the streak plates reflects the improved procedures for sterilizing the coupons prior to testing.

Table 4-54. Liquid Culture Assessment of Coupons Inoculated with *Bacillus anthracis* Ames Spores following Extraction (Peridox)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	-	+	+	+	-	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-55. Liquid Culture Assessment of Coupons Inoculated with *Bacillus subtilis* Spores following Extraction (Peridox)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	-	-	+	-	+	+	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

Table 4-56. Liquid Culture Assessment of Coupons Inoculated with *Geobacillus stearothermophilus* Spores following Extraction (Peridox)

Organism/Test Material	Day 1							Day 7						
	S1	S2	S3	S4	S5	S6	Bl	S1	S2	S3	S4	S5	S6	Bl
Industrial-Grade Carpet														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	-	-	+	-	-	-	ND	+	+	+	+	+	+	ND
Bare Wood														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
Galvanized Ductwork														
Sprayed H_2O	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Sprayed Peridox	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND

S1 = Sample 1
S2 = Sample 2
S3 = Sample 3
S4 = Sample 4
S5 = Sample 5
S6 = Sample 6
Bl = Blank (not inoculated with spores)
ND = Not determined
"+" = growth; "-" = no growth

The results of the decontaminated coupons from the qualitative assessment were consistent with the results from the quantitative assessment and are summarized in Table 4-57. In all cases where growth of spores was observed in the quantitative method, there was a corresponding observation of growth in the liquid culture medium after seven days incubation. In all cases, viable spores were shown to be present in the positive controls using both the quantitative and qualitative methods.

Table 4-57. Summary of Results Obtained from the Quantitative and Qualitative Assessments when Comparing Decontaminated Coupons (Peridox)

Material	*B. anthracis* Ames		*B. subtilis*		*G. stearothermophilus*	
	A	B	A	B	A	B
Industrial-Grade Carpet	+	+	+	+	+	+
Bare Wood	+	+	+	+	+	+
Galvanized Metal Ductwork	+	+	+	+	+	+

A = Quantitative Assessment
B = Qualitative Assessment at seven days
"+" = observed CFU or growth; "-" = no observed CFU or no growth

4.6.2 Damage to Coupons

Before and after decontamination of the test coupons, the decontaminated coupons were visually inspected; and any obvious changes in the color, reflectivity, and apparent roughness of the coupon surfaces were recorded. No damage (e.g., change in surface texture, color) or visible change was observed during this evaluation to any of the test coupons.

4.6.3 Other Factors

4.6.3.1 Operator Control

On each day of testing, the sporicidal technology was prepared fresh according to the vendor's instructions. A NIST-traceable thermometer/hygrometer indicated that the temperature and RH were maintained within the specified range of 22 to 35ºC and <70% RH.

4.6.3.2 Technology Spray Deposition

Gravimetric analysis for total deposition (coupon + run-off) for the sporicidal technology and daily verification of spray performance were performed and are detailed in Section 4.2.3.2. The results of the initial Peridox spray deposition are shown in Table 4-14 for glass and bare pine wood. Daily verification of the amount of spray deposition based on three separate days (N=4 glass coupons per day) resulted in a mean (± SD) mass of 0.46 ± 0.01 grams, which was not statistically different (p = 0.073) than the initial spray deposition on glass coupons of 0.43 ± 0.02 grams.

4.6.3.3 Neutralization Methodology

The approach used for testing neutralization efficiency for Peridox is described in Section 4.2.3.3 and is also described in the test/QA plan.[1] Details of the vendor-recommended neutralizer and selected concentration are shown in Table 4-15.

5.0 Performance Summary

5.1 pH-Amended Bleach Results

- The results of the decontamination tests of the pH-amended bleach varied according to the contaminating biological agent or organism – *B. anthracis* Ames, *B. anthracis* Sterne, *B. subtilis*, or *G. stearothermophilus* – and the porosity of the test coupons. Of the seven test coupons, the four relatively non-porous test materials (glass, galvanized metal ductwork, decorative laminate, and painted wallboard paper) yielded higher log reductions than the test materials considered relatively porous (industrial carpet, bare wood, and painted concrete). In fact, no viable spores could be extracted from glass, galvanized metal ductwork, and decorative laminate following decontamination by pH-amended bleach.

- The decontamination efficacy of amended bleach was high (i.e., 7.2-7.9 log reduction) for hard, nonporous surfaces (glass, decorative laminate and galvanized metal ductwork) and low (0.28-2.0 log reduction) for the porous surfaces (industrial grade carpet, bare wood, and painted concrete) for *B. anthracis* Ames. For *B. anthracis* Sterne and *B. subtilis*, the results were similar; however, for *G. stearothermophilus*, the log reductions were generally lower for hard, nonporous surfaces (0.75-5.90), as well as for porous surfaces (0.02-1.40).

- Statistical analyses comparing the mean spore log reductions of *B. anthracis* with each of the corresponding values for the other organisms revealed statistically significant differences for five of the seven test materials. For industrial carpet, painted wallboard paper, and painted concrete, the log reduction in *B. anthracis* Sterne spores was statistically higher than that of *B. anthracis* Ames. When compared to *B. anthracis* Ames, statistically lower mean log reductions in *G. stearothermophilus* spores were observed on decorative laminate, galvanized metal, and painted wallboard paper. A statistically lower log reduction in *B. subtilis* spores compared to *B. anthracis* Ames spores was observed on painted wallboard paper. However, a statistically higher log reduction in *B. subtilis* spores compared to *B. anthracis* Ames spores was observed on painted concrete.

- To assess whether or not viable spores remained in or on the coupons following decontamination and subsequent extraction (to quantitate extractable, viable spores), both extracted control and extracted decontaminated coupons were placed in tryptic soy broth and incubated for seven days. The contents of the tubes were examined at one and seven days for cloudiness as an indicator of growth. Where no growth was observed using the quantitative extraction method, the qualitative method showed corresponding no growth of residual spores on the extracted coupon.

- In the cases where the liquid cultures exhibited positive growth, a sample of the culture was further analyzed by plating on tryptic soy agar and incubating the plates overnight. For *B. anthracis* Ames, *B. anthracis* Sterne, and *B. subtilis*, the non-sterilized bare wood and painted wallboard coupons yielded mixed microorganism growth in greater than 70% of the streak plates. The percentage of streak plates displaying only growth from the inoculated organism was 40%, 58%, 56%, and 100%

for *B. anthracis* Ames, *B. anthracis* Sterne, *B. subtilis*, and *G. stearothermophilus*, respectively for all coupon types.

- The growth on the non-inoculated decontaminated blanks may have been due to ineffective disinfection (the 70% isopropanol wipe did not sterilize the internal portions of the coupons) prior to inoculating the coupons.

- No visual damage was observed for any of the test coupons subjected to the pH-amended bleach.

5.2 Ten Technologies Evaluated by Screening Test Results

- The results of the decontamination screening tests of the ten sporicidal technologies varied according to the technology employed. All tests were conducted on a glass test coupon contaminated with *B. anthracis* Ames spores.

- Decontamination of the glass test coupons spiked with *B. anthracis* Ames spores using the ten sporicidal technologies resulted in mean log reduction values ranging from 0.37 to ≥7.8.

- After reviewing the draft results, three of the four chlorine dioxide based technology vendors expressed concern that the spray system used in testing may not have been operated optimally for their product. An air pressure of 40 psi was used to atomize the liquid, producing a fine mist (10 – 50 micron diameter droplet size). One vendor suggested that these relatively small size droplets could lead to increased mass transfer of chlorine dioxide from the liquid to gas phase, thus potentially decreasing the chlorine dioxide concentration in the liquid and rendering it less effective. The other two vendors made similar comments. We agree that this phenomenon may be a possibility, although it has not been verified**.** We do note, however, that these three technologies achieved complete inactivation of approximately 10^8 spores in the neutralization tests conducted (solution of spore inoculum + liquid decontamination technology); see Tables 4-18, 4-20, and 4-25.

- The four technologies that exhibited the highest log reductions (see Table 4-13) were selected by the TOPO for further evaluation. The four technologies selected were CASCAD SDF, HI-Clean 605, KlearWater, and Peridox. Results from the further evaluation are outlined in Sections 5.3 through 5.6. These four technologies were also selected because they represent four different types of sporicidal formulations that are available.

- No visual damage was observed for any of the test coupons subjected to any of the ten sporicidal technologies.

5.3 CASCAD SDF Results

- The results of the decontamination tests of CASCAD SDF varied according to the contaminating biological agent or organism – *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* – and the porosity of the test coupons. In general, the relatively non-porous galvanized metal ductwork yielded a much higher log reduction than the two test materials classified as relatively porous (industrial carpet and bare wood).

- Decontamination of the three types of test coupons spiked with *B. anthracis* Ames spores using CASCAD SDF resulted in mean log reduction values ranging from 0.58 to 5.52. Mean log reduction values on all three test materials for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.78 to 4.49 and 0.83 to 4.61, respectively.

- Statistical analyses comparing the mean spore log reductions of *B. anthracis* with each of the corresponding values for the other organisms revealed statistically significant differences for two of the three test materials. For bare wood, the log reduction in *B. subtilis* and *G. stearothermophilus* spores was statistically higher than that of *B. anthracis* Ames. However, for galvanized metal, the log reduction in *B. subtilis* and *G. stearothermophilus* spores was statistically lower than the log reduction in *B. anthracis* spores.

- To assess whether or not viable spores remained in or on the coupons following decontamination and subsequent extraction (to quantitate extractable, viable spores), both extracted control and extracted decontaminated coupons were placed in tryptic soy broth and incubated for seven days. The contents of the tubes were examined at one and seven days for cloudiness as an indicator of growth. In all cases qualitative analysis showed that viable residual organisms were present on the decontaminated coupons (see Tables 4-26, 4-27, and 4-28).

- In the cases where the liquid cultures exhibited positive growth, a sample of the culture was further analyzed by plating on tryptic soy agar and incubating the plates overnight. The percentage of streak plates displaying only growth from the inoculated organism was 100% for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*. This growth in which only the inoculated organism was observed on the tryptic soy agar plates reflects the improved procedures for sterilizing (i.e., gamma irradiation) the coupons prior to testing.

- No visual damage was observed for any of the test coupons subjected to CASCAD SDF.

5.4 HI-Clean 605 Results

- The results of the decontamination tests of HI-Clean 605 varied according to the contaminating biological agent or organism – *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* – and the porosity of the test coupons. Of the three test coupons, the relatively non-porous galvanized metal ductwork generally yielded a much higher log reduction than the two test materials considered relatively porous (industrial carpet and bare wood).

- Decontamination of the three types of test coupons spiked with *B. anthracis* Ames spores using HI-Clean 605 resulted in mean log reduction values ranging from 0.92 to 4.27. Mean log reduction values on all three test materials for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.02 to 2.46 and 0.47 to 1.31, respectively.

- Statistical analyses comparing the mean spore log reductions of *B. anthracis* Ames with each of the corresponding values for the other organisms revealed statistically significant differences for two of the three test materials. For bare wood and galvanized metal ductwork, the log reduction in *B. subtilis* and *G. stearothermophilus* spores was statistically lower than that of *B. anthracis* Ames.

- To assess whether or not viable spores remained in or on the coupons following decontamination and subsequent extraction (to quantitate extractable, viable spores), both extracted control and extracted decontaminated coupons were placed in tryptic soy broth and incubated for seven days. The contents of the tubes were examined at one and seven days for cloudiness as an indicator of growth. In all cases qualitative analysis showed that viable residual organisms were present on the decontaminated coupons (see Tables 4-34, 4-35, and 4-36).

- In the cases where the liquid cultures exhibited positive growth, a sample of the culture was further analyzed by plating on tryptic soy agar and incubating the plates overnight. The percentage of streak plates displaying only growth from the inoculated organism was 100% for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*. This growth in which only the inoculated organism was observed on the tryptic soy agar plates reflects the improved procedures for sterilizing the coupons (i.e., gamma irradiation) prior to testing.

- No visual damage was observed for any of the test coupons subjected to HI-Clean 605.

5.5 KlearWater Results

- The results of the decontamination tests of KlearWater varied according to the contaminating biological agent or organism – *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* – and the porosity of the test coupons. In general, the relatively non-porous galvanized metal ductwork yielded higher log reduction than the two test materials considered relatively porous (industrial carpet and bare wood).

- Decontamination of the three types of test coupons spiked with *B. anthracis* Ames spores using KlearWater resulted in mean log reduction values ranging from 0.05 to 0.92. Mean log reduction values on all three test materials for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.12 to 0.30 and 0.72 to 0.98, respectively.

- Statistical analyses comparing the mean spore log reductions of *B. anthracis* with each of the corresponding values for the other organisms revealed statistically significant differences for all three of the test materials. For industrial carpet, the log reduction in *B. subtilis* and *G. stearothermophilus* spores was statistically higher than that of *B. anthracis* Ames. For bare wood, the log reduction in *G. stearothermophilus* spores was also statistically higher than the log reduction in *B. anthracis* spores. However, for galvanized metal, the log reduction in *B. subtilis* spores was statistically lower than the log reduction in *B. anthracis* spores.

- To assess whether or not viable spores remained in or on the coupons following decontamination and subsequent extraction (to quantitate extractable, viable spores), both extracted control and extracted decontaminated coupons were placed in tryptic soy broth and incubated for seven days. The contents of the tubes were examined at one and seven days for cloudiness as an indicator of growth. In all cases qualitative analysis showed that viable residual organisms were present on the decontaminated coupons (see Tables 4-42, 4-43, and 4-44).

- In the cases where the liquid cultures exhibited positive growth, a sample of the culture was further analyzed by plating on tryptic soy agar and incubating the plates overnight. The percentage of streak plates displaying growth from only the inoculated organism was 100% for *B. anthracis* Ames, *B.*

subtilis, and *G. stearothermophilus*. This growth in which only the inoculated organism was observed on the tryptic soy agar plates reflects the improved procedures for sterilizing (i.e., gamma irradiation) the coupons prior to testing.

- No visual damage was observed for any of the test coupons subjected to KlearWater.

5.6 Peridox Results

- The results of the decontamination tests of Peridox varied according to the contaminating biological agent or organism – *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* – and the porosity of the test coupons. In the case of *B. anthracis* Ames, the decontamination efficacy values for the relatively non-porous galvanized metal ductwork and wood were essentially the same; whereas, industrial-grade carpet yielded values slightly lower than the values obtained for wood and metal.

- Decontamination of the three types of test coupons spiked with *B. anthracis* Ames spores using Peridox resulted in mean log reduction values ranging from 0.87 to 1.05. Mean log reduction values on all three test materials for *B. subtilis* and *G. stearothermophilus* spores ranged from 0.72 to 2.08 and 1.93 to 4.38, respectively.

- Statistical analyses comparing the mean spore log reductions of *B. anthracis* with each of the corresponding values for the other organisms revealed statistically significant differences for all three of the test materials. For industrial carpet and galvanized metal, the log reduction in *B. subtilis* and *G. stearothermophilus* spores was statistically higher than that of *B. anthracis* Ames. For bare wood, the log reduction in *G. stearothermophilus* spores was also statistically higher than the log reduction in *B. anthracis* spores.

- To assess whether or not viable spores remained in or on the coupons following decontamination and subsequent extraction (to quantitate extractable, viable spores), both extracted control and extracted decontaminated coupons were placed in tryptic soy broth and incubated for seven days. The contents of the tubes were examined at one and seven days for cloudiness as an indicator of growth. In all cases qualitative analysis showed that viable residual organisms were present on the decontaminated coupons (see Tables 4-50, 4-51, and 4-52).

- In the cases where the liquid cultures exhibited positive growth, a sample of the culture was further analyzed by plating on tryptic soy agar and incubating the plates overnight. The percentage of streak plates displaying only growth from the inoculated organism was 100% for *B. anthracis* Ames, *B. subtilis*, and *G. stearothermophilus*. This growth in which only the inoculated organism was observed on the tryptic soy agar plates reflects the improved procedures for sterilizing (i.e., gamma irradiation) the coupons prior to testing.

- No visual damage was observed for any of the test coupons subjected to Peridox.

5.7 Comparison of pH-Amended Bleach with Down-Selected Technologies

In general, treatment of inoculated coupons with sprayed pH-amended bleach and the four down-selected technologies yielded higher log reductions on non-porous compared to porous materials. However, one notable exception to this is that sprayed Peridox promoted higher log reductions of *G.*

stearothermophilus on the porous materials (carpet and wood) compared to the non-porous galvanized metal. The spray-applied CASCAD SDF, HI-Clean 605, KlearWater, and Peridox consistently yielded higher log reductions in *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* spores on industrial carpet coupons compared to pH-amended bleach with the exception of KlearWater for *B. anthracis* Ames. Amended bleach performed the best on galvanized metal, for all spores, with the exception of CASCAD SDF against *G. stearothermophilus.* Moreover, log reductions in *B. anthracis* Ames, *B. subtilis*, or *G. stearothermophilus* spores on bare wood coupons sprayed with Peridox were greater than those sprayed with pH-amended bleach or the other technologies.

6.0 References

1. *Technology Testing and Evaluation Program Test/QA Plan for Evaluating Liquid and Foam Sporicidal Spray Decontaminants*, Version 1, Battelle, Columbus, Ohio, March 2006.

2. *Quality Management Plan (QMP) for the Technology Testing and Evaluation Program (TTEP)*, Version 1, Battelle, Columbus, Ohio, January 2005.

3. Rogers JV, Sabourin CL, Taylor ML, Riggs K, Choi YW, Richter WR, Rudnicki DC. BIOQUELL, Inc. Clarus™ Hydrogen Peroxide Gas Generator. Report submitted to EPA Environmental Technology Verification, ETV Building Decontamination Technology Center, March 2004. (http://www.epa.gov/etv/verifications/vcenter11-1.html).

4. Rogers JV, Sabourin CL, Taylor ML, Riggs K, Choi YW, Richter WR, Rudnicki DC. CERTEK® Inc. 1414RH Formaldehyde Generator/Neutralizer. Report submitted to EPA Environmental Technology Verification, ETV Building Decontamination Technology Center, August 2004, (http://www.epa.gov/etv/verifications/vcenter11-2.html).

5. Rogers JV, Sabourin CL, Taylor ML, Riggs K, Choi YW, Richter WR, Rudnicki DC. CDG TECHNOLOGY, Inc. Bench-Scale Chlorine Dioxide Generator: Biological agent Decontamination. Report submitted to EPA Environmental Technology Verification, ETV Building Decontamination Technology Center, September 2004, (http://www.epa.gov/etv/verifications/vcenter11-3.html).

6. Rogers JV, Sabourin CL, Richter WR, Choi YW, Waugh JD, Taylor ML, Riggs KB, Stone HJ, Willenberg ZJ, Krile RT. Evaluation of Sporicidal Decontamination Technology: Sabre Technical Services Chlorine Dioxide Gas Generator. Report submitted to EPA Technology Testing and Evaluation Program, April 2006, (http://www.epa.gov/nhsrc/news/news060506.html).